Mechanism of Passivity Breakdown in Seawater

Final Technical Report

on

Contract No.
N00014-90-J-1947

May 1, 1990 — December 31, 1995

Submitted to

Dr. A. John Sedriks
Materials Division, Code 1131M
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5660

Submitted by

Stephen C. Dexter
Professor
College of Marine Studies
University of Delaware
Lewes, DE 19958

UNIVERSITY OF DELAWARE

Mechanism of Passivity Breakdown in Seawater

Final Technical Report

on Grant No.
N00014-90-J-1947

May 1, 1990 to December 30, 1995

Submitted To:

Office Of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5660

Attention:
Dr. A. John Sedriks
Materials Division, Code 1131M

Submitted by:

Stephen C. Dexter
Professor
College of Marine Studies
University of Delaware
Lewes, DE 19958

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April, 1997	Final Report

4. TITLE AND SUBTITLE

Mechanism of Passivity Breakdown in Seawater

5. FUNDING NUMBERS

Grant No.

N00014-90-J-1947

6. AUTHOR(S)

Stephen C. Dexter

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES)

College of Marine Studies
University of Delaware
Lewes, DE 19958

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAMES(S) AND ADDRESS(ES)

Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5660

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 words)***

Natural assemblage biofilms developed on passive metals in seawater are complex in structure and diverse in bacterial community. The mechanism by which such biofilms change the chemistry at the metal surface and ennoble the open circuit potential (OCP) on platinum and stainless steels has been studied. The chemistry responsible for ennoblement in our biofilms has been shown to involve highly variable and synergistic combinations of peroxide (low mM range), acidic pH, oxygen (generally below 1 ppm) and the heavy metals, iron and manganese (mM range). Chemical simulation experiments demonstrated that some dissolved oxygen in the bulk solution was necessary for ennoblement to occur. Other experiments demonstrated that an acidic pH at the metal-biofilm interface is necessary for the observed amount of ennoblement on platinum to be achieved in the presence of peroxide. Thermodynamic factors (reaction potentials and pH) were found to be the major contributors to ennoblement on platinum, whereas the kinetic factors, exchange current density and Tafel slope, also contributed on stainless alloy S44660. No systematic relation between passive current density and ennoblement was found.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Passivity, Corrosion Initiation, Biofilms, Ennoblement, Seawater, peroxide, heavy metals, Bacterial Metabolism, Interfacial Chemistry	87
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
unclassified	unclassified	unclassified	UL

Table of Contents:

Introduction

This report is organized into three chapters, each based on papers written by Chandrasekaran, Dexter and Luther (Chapter 1) or Chandrasekaran and Dexter (Chapters 2 and 3). All of the data included has been presented by Chandrasekaran or Dexter at National and International meetings, and it appears in the following publications resulting from the grant:

S. C. Dexter, 1992, "Implications of Biological Effects to Localized Corrosion of Stainless Alloys," Proceedings 1st Pan American Congress on Corrosion and Protection, Mar del Plata, Argentina, October, 1992, p. 485-500.

P. Chandrasekaran and S.C. Dexter, 1993, "Mechanism of Potential Ennoblement on Passive Metals by Seawater Biofilms," Paper No. 493, CORROSION/93, NACE International, Houston, TX.

S. C. Dexter, P. Chandrasekaran, H-J Zhang and S. Wood, 1993, "Microbial Corrosion in Marine Environments: Effect of Micro-fouling Organisms on Corrosion of Passive Metals", Proc. 2nd USA/Argentina Workshop on Biocorrosion and Biofouling, Buckman Laboratories Intl., Inc., Memphis, TN, pp. 171-180.

R.G.J. Edyvean and S. C. Dexter, 1993, "MIC in Marine Industries," in Microbiologically Influenced Corrosion, A Practical Manual, G. Kobrin, Ed., NACE International, Houston, TX, pp. 47-63.

P. Chandrasekaran and S.C. Dexter, 1993, "Factors Contributing to Ennoblement of Passive Metals Due to Biofilms in Seawater," Proc. 12th International Corrosion Congress, Vol. 5B, NACE Internatinal, Houston, TX, pp. 3696-3707.

S. C. Dexter, H-J. Zhang and P. Chandrasekaran, 1994, "Biofouling Effects on Corrosion of Stainless Alloys in Seawater," Biotoxins, Biodegradation and Biodeterioration Research IV, Plenum Press, pp. 596-617.

P. Chandrasekaran and S. C. Dexter, 1994, "Bacterial Metabolism in Biofilm Consortia," Paper # 276, CORROSION/94, NACE, International, Houston, TX.

P. Chandrasekaran and S. C. Dexter, 1994, "Thermodynamic and Kinetic Factors Influenced by Biofilm Chemistry Prior to Passivity Breakdown," Paper # 482, CORROSION/94, NACE, International, Houston, TX.

S. C. Dexter, 1994, "Mechanism of Ennoblement by Biofilms on Active/Passive Alloys Immersed in Seawater," in Proc. Tri-Service Conference on Corrosion, June, 1994, Orlando, FL.

S. C. Dexter, 1995, "Effect of Biofilms on Marine Corrosion of Passive Alloys," in Bioextraction and Biodeterioration of Metals, C. Gaylarde and H. Videla, Eds., Cambridge University Press, pp. 129-168.

Chapter 1

Presence of Peroxide in Marine Biofilms and Its contribution to Potential Ennoblement on Passive Metals

Abstract

Platinum metal coupons were used in studying the mechanism of ennoblement of open circuit potential (OCP) due to marine biofilms. Presence of peroxide in the low millimolar range (possibly as a by product of bacterial metabolism) has been verified in biofilms formed in two widely separated geographical locations. Experiments with enzymes indicated that some dissolved oxygen was necessary in the bulk solution for ennoblement to occur. Abiotic simulation experiments demonstrated, however, that an acid pH at the metal-biofilm interface is also necessary for the observed amount of ennoblement on platinum to be achieved in the presence of peroxide. The possibility of pH below 3 in marine biofilms was indicated by growing organisms from the film on agar media containing pH indicating dyes. It is suggested that one mechanism for ennoblement involves a synergistic effect of peroxide and acid pH at low oxygen within the biofilm near the metal surface.

Key words: Ennoblement, peroxide, interfacial chemistry, bacterial metabolism, passive, seawater.

Introduction

Biofilm formation in natural waters over different substrata is a well studied phenomenon (Corpe, 1970). However, the interfacial chemistry changes produced by bacterial attachment and colonization are challenging to study due to the small dimensions of the biofilm, intriguing complexity of marine biofilm consortia and metabolism, and the lack of in-situ analytical techniques to suit the dimension of the biofilm (Characklis and Wilderer, 1989). It is well documented that biofilms from natural waters of all salinities ennoble passive metal corrosion potentials (Mollica and Trevis, 1976; Johnsen and Bardal, 1985; Johnsen and Bardal, 1986; Mollica et al., 1988; Dexter and Gao, 1988; Scotto et al., 1985; Scotto, 1988; Mollica, 1992; Salvago et al., 1992; Eashwar et al., 1993). There have also been a few published instances where ennoblement did not occur (Mansfeld and Little, 1991; Mansfeld et al., 1992). It is firmly believed that the interfacial chemistry changes are directly responsible for this ennoblement phenomenon.

An extensive literature review (Dexter and Lin, 1988; Chandrasekaran and Dexter, 1993a; Buchanan et al., 1989; Chandrasekaran and Dexter, 1993b; Van den Brink et al., 1980) suggests that potential ennoblement in the presence of biofilms could be a result of enhancement of the oxygen reduction reaction by 1) a decrease in pH, 2) macrocyclic organo-metallic catalysis or 3) bacterially produced enzymatic catalysis. Dexter and Lin (1988) and Buchanan et al (1989) have put forward a mechanism based on lowering of pH in a biofilm. Lowering of pH within a biofilm is thought to be a major contributor for ennoblement because the potential of the oxygen reduction reaction shifts noble by 59 mV for each pH unit decrease (Pourbaix, 1974). But the reasons for pH decrease within a natural population biofilm formed in seawater are not well understood. However, recent studies in our laboratory indicate that biofilms formed over platinum coupons accumulate heavy metals like iron and manganese due to bacterial metabolism (Chandrasekaran and Dexter, 1993b; Chandrasekaran and Dexter, 1994a). The accumulated heavy metals can undergo hydrolysis and contribute to decrease in pH (Chandrasekaran, 1995). However, substantial decrease in pH can also occur due to acid producers, secretion of extracellular acidic polymers, or other reactions like oxidation of reduced bacterial metabolites (sulfides or organic compounds) by oxygen or peroxide (Millero et al, 1989; Luther, 1990).

Recently, a new hypothesis for ennoblement mechanism has been put forth by Mollica (1992) and Eashwar et al., (1993) in which the biofilm pH is considered to be neutral for ennoblement. Mollica (1992) measured the OCP of stainless steel in seawater acidified down to pH 5. Since the OCP did not rise to the level observed for biofilms, he ruled out the possibility of acidification as a mechanism for ennoblement. Mollica (1992) hypothesized that ennoblement is due to depolarization of the cathodic oxygen reaction by bacterially produced catalytic enzymes with a maximum efficiency at pH near 8. Eashwar (1993) pointed out that bacterially produced enzymes are active only at near neutral pH, and he hypothesized that the siderophores produced by bacteria would be important to ennoblement.

Oxygen reduction by macrocyclic organo-metallic catalysis (Johnsen and Bardal, 1985; Van den Brink, 1980; Yeager, 1985) using porphyrins, phthalocyanins and tetraazaannulenes in electrochemical fuel cells (Vanden Brink, 1980; Hancock and Martell, 1989) does not take place instantaneously at low temperatures as a four electron reduction, but predominantly proceeds

through a single or two electron reduction process with peroxide as an intermediate. Other factors such as electrode surface coverage by the catalyst (Bettelheim et al., 1980; Ozer et al., 1989; Bettelheim et al., 1987; Anson et al., 1985), differences in central metal atom and the substitution on the porphyrin ring (Tarasevich et al., 1977; Kobayashi and Nishiyama, 1984; Kuwana et al., 1978; Yeager, 1976), electron transfer rate from the electrode surface to the catalyst (Saveant and Vianello, 1963; Bettelheim et al., 1979; Anson, 1983; Therien et al., 1991), and solubility of the catalyst (Kuwana et al., 1978; Forshey and Kuwana, 1983; Bettelheim and Kuwana, 1979; Chan et al., 1985) are thought to be contributing reasons for peroxide production. Yeager (1986) explained that conformational difference in the orientation of catalyst on the electrode surface can also give rise to peroxide production. However, a four electron oxygen reduction is also possible using a specifically engineered catalyst molecule like dicobalt cofacial porphyrin dimer (Collman et al., 1981). Porphyrins are present in the respiratory system of bacteria (Brock and Madigan, 1988) and can be anticipated to be released into the biofilm matrix due to cell lysis (Lock et al., 1984). These porphyrin compounds can participate in oxygen reduction electrocatalysis. If this is true, then it can be anticipated that peroxide is produced as an intermediate due to abiotic oxygen reduction in a predominantly biotic system. The contribution to peroxide production can be significant as the biofilm matures and releases a substantial quantity of porphyrin in the biofilm. Presence of Peroxide in marine biofilms was speculated by Salvago et al., (1992) as a reason for ennoblement.

The biotic component of peroxide production can come from the bacterial metabolism itself. Microorganisms have different requirements of, and tolerance for oxygen. Bacterial respiration produces various forms of oxygen reduction products that are toxic to the organisms (Brock and Madigan, 1988; Price and Morel, 1990; Gottschalk, 1986). In order to deal with the toxic products, bacteria have three enzymes, catalase, peroxidase and superoxide dismutase (Brock and Madigan, 1988; Price and Morel, 1990; Gottschalk, 1986; Morris, 1984). These enzymes are also porphyrin type compounds. Various groups of microorganisms have different levels of enzyme activity. Aerobic microorganisms have all three enzymes, whereas obligate anaerobes lack one of the three enzymes, contributing to their inability to function in aerated environments (Gottschalk, 1986). The enzyme system works in a coordinated fashion as shown below and controls the concentrations of toxic forms of oxygen (Gottschalk, 1986).

Superoxide Dismutase	$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$	(1)
Catalase	$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$	(2)
Peroxidase	$H_2O_2 + NADH + H^+ \rightarrow 2H_2O + NAD^+$	(3)

Where NADH and NAD^+ are reduced and oxidized forms of nicotinamide adenine dinucleotide.

The superoxide dismutase enzyme breaks down superoxide free radical into peroxide and oxygen, whereas peroxidase and catalase limit the peroxide concentration. Of the toxic compounds produced above, peroxide is a well known oxidizer. In a natural assemblage biofilm, it can readily be seen that the enzyme system will limit the concentration of the toxic products. It can also be anticipated that in a dynamic biofilm, respiration of microbes will always be producing peroxide, and the enzyme system will be controlling the concentration. This would give rise to a steady state concentration of peroxide in a biofilm. Inhibition of the enzyme, catalase, resulted in an appreciable concentration of peroxide in a culture of the marine bacterium, *Bacillus* 29 (Ghiorse, 1988). In seawater, a concentration of 200 nM peroxide (Palenik et al., 1988) is produced by photochemical reactions. One study of ground water in sand columns (Britton, 1985) showed significant toxicity toward inoculated bacteria at H_2O_2 concentrations above $1.5X10^{-2}$ M.

The possibility of peroxide formation as a contributor to ennoblement in natural population biofilm has been neglected in previous studies. In the present work the contribution of H_2O_2 towards ennoblement was investigated. Two sites were selected for carrying out the ennoblement mechanistic studies. One site was on the lower Delaware Bay, at College of Marine Studies (CMS) Facilities at Lewes, DE and the other one was a tropical environment at Offshore Platform and Marine Electrochemistry Center on the Gulf of Mannar, a unit of Central Electrochemical Research Institute at Tuticorin, India.

Experimental Procedure

Since enhancement of the cathodic reduction reaction by acidic pH and peroxide are suspected to be contributors to ennoblement, experiments were conducted to account for these two factors. Platinum metal electrodes were used (total area of each coupon was approximately 9 cm^2) to obtain a relatively simple oxygen electrode system for experimental investigations. Platinum wire electrical leads were attached to the platinum coupons by spot welding. A nickel wire was further spot welded to the platinum lead. The platinum nickel junction was kept dry by encasing it in a glass tube with the lower end of the tube sealed to the platinum wire section. The open circuit potentials of these coupons were measured using a high impedance digital voltmeter, with SCE for reference, and reasonably stable ennoblement was considered to be indicative of a mature biofilm.

Biofilms were developed on a series of 600 grit polished platinum coupons using once-through flowing seawater at the College of Marine studies, University of Delaware research facilities on the Delaware Bay at Lewes. The seawater characteristics during the exposure were: temperature 22 to 26^{0}C, pH 7.7 to 8.1, salinity 25 to 31 parts per thousand and oxygen air saturated at 5 to 8 ppm and H_2O_2 below the detection limit of the peroxidase enzyme strip. This water has typically grown 30 to 160 mm thick biofilms (Dexter and Zhang, 1991) with macroscopically complete coverage. Due to the thickness of these films, it is usually impossible to count all the microorganisms. Microscopic examination in the epifluorescence microscope at 1000X after staining with DAPI reveals a diverse microflora ranging from coccoidal bacteria to short and curved rods to filamentous forms. The concentration of organisms (Dexter and Zhang, 1991) in the surface layers of the film varies from 3 x 10^5 to 2 x 10^6 cells cm^{-2} of coupon surface. with significantly higher numbers in microcolonies. The biofilms grown in this study were not specifically analyzed, but were assumed to be similar to the above. The water characteristics at Tuticorin during the exposure were: temperature 25.6 to 27.4^{0}C, dissolved oxygen 6 ppm, salinity 33.2 to 33.9 parts per thousand and H_2O_2 below the detection limit of the peroxidase enzyme strip. Biofilms formed in Tuticorin waters on platinum in the present studies were not analyzed. It is expected, however, that they should be similar to those already characterized from those same waters by Maruthamuthu, et al., (1993). They found that the ratio of heterotrophic bacteria to thiosulfate oxidizing bacteria was high initially, but decreased significantly as the biofilm matured.

At Delaware, electrochemical experiments were conducted using an IBM computer with PARC 342 software controlled 273A potentiostat (EG & G, Princeton Applied Research) with standard accessories and cell set up. This set up was used for cathodic polarization of platinum electrodes with and without biofilm, and for E_{corr} measurements at various combinations of pH, peroxide and oxygen. Cyclic voltammetry was carried out using IBM Instruments Inc. EC 225 Voltammetric Analyzer with Hewlett Packard Moseley Division X-Y recorder. The voltammograms from the X-Y recorder were scanned directly into a Macintosh computer with Canvas 2.1 software and printed on a laser printer. Platinum was used for working and counter electrodes, and SCE for reference. During all the experiments, measurement of dissolved oxygen was carried out using a YSI model 57 oxygen meter and the pH was measured using Fisher digital pH meter.

Experiments in which the inorganic bulk water chemistry was varied were carried out to simulate the conditions thought to exist in a biofilm. The trial and error method was adopted to verify the oxidation and reduction peaks that were produced. Once the peaks were identified as oxygen reduction and peroxide oxidation, identification of peroxide in a biofilm was carried out using peroxide indicator strips bought from E. Merck. Peroxidase enzyme transfers oxygen from peroxide to an organic redox indicator, which is then converted to a blue colored oxidation product. The concentration of peroxide present is determined by comparing the intensity of the blue color to a standard color chart. The minimum and maximum detectable limits of peroxide concentration using this technique are 0.5 ppm (0.014 mM) and 25 ppm (0.74mM) respectively. The indicator strips were rubbed over an area of approximately one square centimeter on the biofilmed coupons, and the color of the strip was matched to the chart for the concentration. This amount was compared with the abiotically added peroxide. An independent set of experiments was also conducted to identify the presence of peroxide in biofilms. This experiment consisted of formulating a solution of colorless ferrothiocyanate which would turn red in the presence of peroxide. Concentrations of peroxide down to about 0.02 mM can be identified using this technique, with the concentration being proportional to the intensity of the red color. A calibration procedure using this indicator was also carried out in the laboratory to find out the approximate concentration range of peroxide in the biofilms.

Another series of experiments was carried out to determine the effect of decreasing the peroxide concentration on the OCP of biofilmed electrodes. In order to do this, beef liver catalase (Sigma Chemical Co.) at a concentration of 0.5 mg ml^{-1} was added to the seawater surrounding the ennobled electrode. The catalase added should dismute any peroxide with which it comes into contact. The hope was that through a combination of peroxide diffusion out of the biofilm into the bulk solution and catalase diffusion into the biofilm, the peroxide concentration would be reduced enough to affect its contribution to ennoblement. Similar concentrations of both active and deactivated (control) catalase were added. The deactivated catalase control was achieved by exposure to sunlight under aerated conditions (Mitchell and Anderson, 1965). Seawater with catalase was exposed for two hours to artificial sunlight produced by Phillips cool-white florescent lamps at an irradiance of 900 mEinsteins m^{-2} sec^{-1}. Enzyme activity (Worthington Manual, 1972) was measured using a Bausch and Lomb spectrophotometer operating at 240 nm and connected to a strip chart recorder. A calibrated level of absorbance was first established for 30% peroxide with 7.5 pH, 1M NaCl and 0.1M Na_2HPO_4 buffer in quartz cuvettes. When active enzyme was added, absorbance decreased with time, while additions of deactivated enzyme produced no change.

In order to determine the level of acidity that various organisms present in our natural assemblage biofilms could produce, microbiological agar plating was carried out using Difco Marine Agar 2216 Medium containing recommended concentrations of various pH indicating dyes (Sigma) (Costilow, 1981). For this experiment alone biofilms were formed on coupons of stainless alloy UNS S44660 (nominal composition in percent by weight: Cr 27.36, Ni 2.02, Mo 3.53, C 0.25, bal Fe). Once formed, biofilms were scraped from the metal coupons into 100 ml of sterile seawater and gently agitated to disperse the organisms. Samples of this seawater were then inoculated with liquid agar medium containing the appropriate pH indicating dyes in petri dishes and allowed to solidify. Each pH indicating dye had a characteristic color change corresponding to a range of approximately two pH units. For example, phenol red is yellow at pH below 6.8 and red above pH 8.2. The color shift from yellow to red takes place progressively as one proceeds from pH 6.8 to 8.2.Four petri dishes were used for each pH range. Deaeration for biofilm simulation studies was carried out using nitrogen sparging, and the pH of the bulk seawater was adjusted when necessary using either 0.1N HCl or 0.1N NaOH.

Results

Open circuit potential (OCP) values and H_2O_2 concentrations were measured for biofilmed platinum at the Delaware and Tuticorin locations in natural seawater. Platinum OCP values are shown in Figure 1.1. For mature biofilms from Delaware Bay seawater, peroxide concentrations covering the whole detectable range from 0.14 to 0.73 mM were measured using the enzyme strips. During the initial stages of biofilm formation at Tuticorin, the highest concentration measured was 0.28 mM. Enzyme strips did not show any color for the natural seawater sample, probably because the concentration of peroxide naturally occurring in seawater was below the detection limit. Peroxide presence was further confirmed by the ferrothiocyanate test. The reagent turned pale red in the laboratory in the presence of 0.25 mM peroxide. In contrast, it usually turned bright red when a few drops were placed on a mature, ennobling biofilm. This test verified that there was often more than 0.25 mM peroxide in the films tested from Delaware Bay waters.

Typical voltammograms of a biofilmed electrode for various scan rates are shown in Figure 1.2. The voltammogram has one peak each in the forward and reverse scans. The range of potentials scanned was selected to include that for ennoblement but exclude hydrogen evolution, which takes place well below -400 mV SCE. The cyclic voltammetry convention was used in determining that the peak in the forward scan was an oxidation and that in the reverse scan a reduction. There was no direct information from the voltammogram regarding what these oxidation and reduction reactions might be. However, since the experiment was conducted in well aerated natural seawater it was expected that these redox reactions should be part of the oxygen system. The voltammograms show that the reactions are irreversible, which is indicative of the oxygen and peroxide electrode reaction. Figure 1.2 also shows the effect of scan rate on the position of the peaks in the voltammogram. A faster scan rate shifts the oxidation peaks in the positive direction. The shift indicated that the reactions were dependent upon the electron transfer from the substratum. This shows that the substratum will play a role in determining the position of the oxidation and reduction peaks of the voltammogram. Besides substratum properties, the biofilm properties can also play a role in electron transfer. In all the voltammograms that follow, a uniform scan rate of 50 mV/sec was adopted in order to be able to compare voltammograms.

The characteristic cyclic voltammogram shown in Figure 1.3 was produced with a scan rate of 50 mV/sec over the potential range from -400 to + 600 mV SCE. Curves 1 and 2 in this Figure, represent two successive runs on the same biofilmed platinum electrode, whereas curve 3 represents the response of a bare electrode in natural seawater. In all runs with biofilmed platinum coupons the oxidation peak at about 0.35 V SCE in the forward scan and the reduction peak at - 0.15 V SCE in the reverse scan were observed to be reproducible. Curves 1 and 2; produced from the same biofilmed electrode in quick succession show a difference in peak height. This indicated that the chemical species producing the peak in the voltammogram was consumed during the measurement.

Abiotic cyclic voltammetry experiments conducted with a bare platinum electrode with and without oxygen (less than 0.2 ppm (6mM)) are shown in Figure 1.4. The peak in the reverse scan in curve 2 (air saturated) shows that it is oxygen reduction. Curve 1 (deaerated) shows no peak in either forward or reverse scans. This experiment proved that the peak in the reverse scan was due to oxygen reduction.

Abiotic experiments on freshly prepared bare platinum electrodes were carried out to simulate the cyclic voltammogram of biofilmed platinum. Various combinations of oxygen, pH and peroxide were used. Peroxide additions were made by adding appropriate volumes of 30% peroxide solution to one liter of seawater, and the concentration was expressed in mM units using the specific gravity of 30% peroxide as 1.1122 (Washburn, 1928). Typical electrode responses for several experimental conditions are shown in Figure 1.5. Curve 1 represents the response of a platinum electrode in natural seawater deaerated to less than 0.5 ppm (15 mM) of oxygen at pH 2.93 (adjusted by adding HCl). Curve 2 represents the response due to addition of 0.48 mM hydrogen peroxide at pH 2.93, and curve 3 represents 1.44 mM hydrogen peroxide at the same pH. Note in curve 3 that the oxidation peak at 0.35 V SCE is beginning to appear. However, the voltammogram of the biofilmed platinum coupon from Figure 1.3 was not fully reproduced until the seawater was deaerated to an oxygen concentration below 0.5 ppm (15 mM), with 2.4 - 2.9 mM hydrogen peroxide added at pH 2.8 - 2.9 as shown in Figure 1.6. Therefore it is tentatively concluded that the peak at 0.35 V is peroxide oxidation.

The measured peroxide concentrations in the biofilms using peroxidase enzyme strip were less than, but still in the same low millimolar range as that which was added to produce the voltammogram in Figure 1.6. Having identified presence of peroxide in a biofilm, the peak that appears in the forward scan in Figure 1.6, at about 0.35 V SCE is interpreted as oxidation of peroxide to oxygen and the peak that appears in the reverse scan close to -0.15 V SCE as reduction of oxygen.

Based on the above results, an independent set of experiments was carried out to understand more about the synergistic contributions of peroxide and pH towards ennoblement of the OCP. Based on the cyclic voltammetry experiments, an independent set of experiments was carried out to determine the OCP of platinum in deaerated Delaware Bay seawater (0.55 ± 0.05 ppm (17 ± 1 mM) O_2) at various combinations of pH and peroxide. Condition 1 represents the OCP of platinum at pH 8.3 (Figure 1.7). A decrease in pH to 5 (condition 2) produced a jump in OCP to over 300 mV (Figure 1.7) as would be expected from the Nernst equation (Chandrasekaran and Dexter 1993a). Addition of 2.4 mM peroxide at pH 5 gave no further increase in potential (condition 3) (Figure 1.7). A further decrease in pH to 2.9 without peroxide addition increased the potential to about 350 mV (condition 4, Figure 1.7), but it was not until a combination of pH 2.9 and 2.4 mM peroxide was tried that the potential of over 400 mV observed on biofilmed platinum was obtained (condition 5, Figure 1.7).

The deaerated simulation is relevant to the biofilmed case, where the aerobic bacteria would reduce the oxygen concentration. Since peroxide in this system can be produced only through the reduction of oxygen we assumed that production of peroxide took place in the oxic portions of the biofilm. The peroxide thus produced in the oxic portions of the biofilm was transported to the biofilm-substratum interface (Center for Biofilm Engineering NEWS letter, 1994) through the channels present in a biofilm, or through the diffusion process. This creates a scenario in which the biofilm-substratum interface is low in oxygen and some peroxide is also present. This chemical combination will contribute to ennoblement very similar to the result of the above experiment (Figure 1.7) under low oxygen and peroxide concentrations.

To determine the effect of large concentrations of peroxide in a biofilm, an abiotic experiment was carried out. In this experiment, a large volume of peroxide was added to natural seawater under aerated and deaerated conditions and the effect was studied on the OCP of a bare platinum coupon. The OCP values of platinum shifted active in aerated condition, whereas it shifted noble in the deaerated condition Figure 1.8. The conclusions that can be derived from this experiment are that the peroxide in a biofilm can contribute to ennoblement only when the oxygen concentration is low, and if for some reason peroxide is present together with higher oxygen in a biofilm then the OCP will shift active.

The effect of combinations of peroxide and pH under oxygen super-saturation (more than 20 ppm (>0.6 mM)) on platinum is shown in Figure 1.9. A decrease in solution pH caused a noble shift in OCP of platinum in oxygen saturated solutions similar to its effect already demonstrated for deaerated solutions. The addition of peroxide in oxygen saturated solutions, however, caused an active shift in OCP for platinum. The same type of results were also obtained by Hoare (1968). The full amount of ennoblement observed on platinum under natural biofilms could only be reproduced when the proper concentration of peroxide was added to deaerated, low pH solutions.

Cathodic polarization curves were measured for platinum coupons with and without biofilms in natural Delaware Bay seawater (Figure 1.10). The biofilmed coupon showed two Tafel regions separated by a depletion region as compared to the single Tafel region on the bare electrode. These data strongly suggest that there are two reduction reactions taking place under the biofilm. The chemical species responsible for the initial Tafel region becomes depleted as already mentioned by Chandrasekaran and Dexter (1993a).

To identify the importance of peroxide in changing cathodic kinetics, cathodic polarization of bare platinum electrodes with and without peroxide added to the bulk solution was carried out. The first run was done in deaerated natural seawater, while the second run was carried out with natural seawater deaerated using nitrogen (0.5 ppm (15mM) oxygen) and 2.4 mM of peroxide added. Within experimental error, the slopes for oxygen (0.11 V/decade of current) and peroxide (0.12 V/decade of current) were the same (Figure 1.11). The exchange current densities as determined by using the back extrapolation method (Jones, 1992) were 7.9 X 10^{-4} A/m^2 for oxygen

reduction and 3.8 X 10^{-2} A/m^2 for peroxide reduction. This showed that the exchange current density for peroxide reduction was two orders of magnitude higher than that for oxygen. The OCP in the two runs is roughly the same because of the effect of deaeration canceling the effect of peroxide addition in run 2.

A typical catalase enzyme activity measurement using spectrophotometry is shown in Figure 1.12. The decrease in absorbance over time after adding active catalase is directly related to dismutation of H_2O_2 by the active enzyme. In contrast, no decrease in absorbance is noted upon addition of the inactivated enzyme. The decay in OCP values of ennobled platinum electrodes exposed to active and inactive forms of catalase was also recorded. Due to dismutation of H_2O_2 by active catalase, OCP values were expected to drop more rapidly upon active catalase exposure than inactive catalase exposure. However, the rates of decay in OCP values were comparable in both exposures (Figure 1.13).

To understand more about this anomaly, solution chemistry and catalase activity were measured periodically. Hourly measurements of catalase activity showed that the enzyme remained active for nearly 10 hours in seawater. This indicated that the decay in OCP for the first 10 hours after addition of active enzyme could be taken as due to dismutation of H_2O_2. A closer look at the inactive enzyme experiment revealed that the enzyme precipitated out of solution and formed a thin continuous film on the surface of the solution. Active enzyme did the same thing after the first 10 hours. Further experiments were conducted using an ennobled biofilmed coupon exposed to active catalase enzyme. Periodic measurements of OCP, bulk solution pH and dissolved oxygen were recorded. At first the potential decreased with no change in dissolved oxygen (Figure 1.14). As the 10 hour limit approached, however, the dissolved oxygen fell rapidly. This corresponded to the active-inactive transition of the enzyme and the formation of the surface film. Subsequent immersion of catalase treated coupons in natural seawater brought the OCP back to their original ennobled values, after a period of time. This was attributed to a recovery of normal biofilm chemistry.

Figure 1.15 shows the shift in OCP of the oxygen electrode on platinum in air saturated Delaware Bay seawater for the pH range of 2 to 10, along with the theoretical curve (59 mV per pH

unit) calculated from the Nernst equation. As expected, the measured values are lower than those predicted by thermodynamics. The slope of the measured curve was 36.6 ± 0.74 mV per pH unit.

Results from the experiments in which scrapings from our natural assemblage biofilms were mixed with agar incorporating pH indicating dyes are shown in Table 1.1. The color developed by the colonies of microorganisms that grew up in the agar was taken to indicate the pH of the metabolites produced. Note that about one third of the colonies that grew in the agar with bromophenol blue were yellow in color, indicating that the pH was less than three. None of the colonies that grew gave an indication of pH greater than 6.4, the pH above which chlorophenol red turns red. These data do not prove it, but they are consistent with the idea that the pH within a natural marine biofilm could be in the acid range.

Discussion

Figure 1.1 showed potential ennoblement of platinum coupons due to biofilm formation over time. A steady ennobled potential was taken to indicate formation of a mature biofilm. Figure 1.1 showed the OCP values for platinum in Tuticorin waters to be considerably lower than those in Delaware. The reasons aren't very clear. Since the duration of exposure was only 20 days at Tuticorin the biofilms probably weren't mature enough to give a maximum concentration of H_2O_2, and thereby a higher OCP value. The microbial population itself may be widely different between these two locations contributing to this low amount of ennoblement. However, identification of measurable quantities of H_2O_2 at both locations is considered to be significant. It has already been shown that the amount of ennoblement thermodynamically possible on a reversible oxygen electrode in seawater due to a decrease in pH was less than that predicted for the 4 electron reaction by the Nernst equation (Chandrasekaran and Dexter, 1993a). Since all the observed ennoblement on stainless steel particularly in low salinity waters cannot be explained by pH alone (Dexter et al., 1993), other contributory factors were also investigated.

In well aerated seawater, the predominant cathodic reaction on a passive metal surface is oxygen reduction. It is important to understand the oxygen reduction process and the products formed to account for ennoblement. The oxygen reduction as catalyzed by organometallics and

enzymes is usually a single electron transfer rather than a four electron transfer process (Brock and Madigan, 1988; Vasudevan et al., 1990). It was also reported that any aerobic organism would produce a small concentration of peroxide (Fridovich, 1974; Brock and Madigan, 1988). Sun light excites the organic molecules that are present in open ocean water, subsequently producing superoxide free radical (Zepp et al., 1977, 1985; Haag and Hoigne, 1986). Superoxide free radical is also known to be the precursor for peroxide production in many biotic systems (Cooper and Zika, 1983; Cooper et al., 1988; Zika et al., 1985; Zaifirou et al., 1984). Biofilms formed over metallic substrata are complex media in which organometallic catalysis, enzymatic reduction of oxygen, electrochemical oxygen reduction and some photochemical reactions (Cooper and Zika, 1983) depending on the available light levels can all take place simultaneously giving rise to peroxide production. With this basic understanding, experiments were conducted in this paper to identify and measure the peroxide present in the biofilms.

As a first step in the measurement of peroxide in biofilms, experiments were conducted to simulate the chemistry thought to exist under a biofilm. Simulation of biofilm-substratum interfacial chemistry and reproduction of signature cyclic voltammograms of a biofilmed ennobled platinum electrode lead to further work on identification and concentration measurement of peroxide in marine biofilms (see Figures 1.2 to 1.6). Subsequent to simulation of voltammograms, the presence of peroxide in biofilms was verified using the peroxidase enzyme strip; 0.14 to 0.73 mM range of peroxide was measured. Since enzyme tests are highly specific, the presence of peroxide in the biofilm was indicated without ambiguity.

Peroxide was also measured in the range of 0.14 to 0.28 mM in the biofilms developed at the Tuticorin site in India. This was an important result because peroxide was found in biofilms formed at a different location. If bacterial metabolism is the primary producer of peroxide (Ghiorse, 1988) in marine biofilms, then presence of peroxide can be expected to be widespread in oxic biofilms. The concentrations reported for the Tuticorin site were not as high as that for Delaware site. Probably, this was one of the main reason for lower levels of ennoblement for samples reported for Tuticorin compared to the Delaware site. However, the bacterial population differences between Delaware and Tuticorin can be another reason for this difference in the level of ennoblement.

Peroxide was further confirmed by the ferrothiocyanate test, which showed that there was more than 0.25 mM peroxide within the biofilm on a platinum electrode ennobled to over 400 mV SCE. The measured peroxide concentrations in the biofilms using peroxidase enzyme strip were less than, but still in the same low millimolar range as that which was added to produce the voltammogram in Figure 1.7. Having identified the presence of peroxide in a biofilm, the peak that appears in the forward scan in Figure 1.2 (scan rate 50 mV/sec), at about 0.35 V SCE is interpreted as oxidation of peroxide and the peak that appears in the reverse scan close to -0.15 V SCE as reduction of oxygen. The oxidation of peroxide (Pourbaix, 1974) is electrochemically driven due to potential scanning in cyclic voltammetry according to:

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \quad (4)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (5)$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad \text{(Overall reaction)} \quad (6)$$

The cyclic voltammograms from biofilmed samples in Figure 1.2 showed that both oxidation and reduction reactions took place during the potential scan. Since oxygen is the primary redox system involved in cathodic reaction in aerated natural waters, the peaks in the voltammograms were probably associated with the various oxygen reaction products. Theoretical concepts indicate that peroxide is the most likely reaction product (Van den Brink et al., 1980; Hancock and Martell, 1989; Bettelheim et al., 1980; Tarasevich et al., 1977; Yeager, 1976; Therien et al., 1991; Chan et al., 1985; Yeager, 1986) and the cyclic voltammetry experiments are consistent with that idea. The presence of peroxide in biofilms formed over widely separated geographical location is also significant. This is important to establish because the standard potential for the peroxide reduction reaction is more noble than that for oxygen reduction (see equations 7 and 8 below) (Hoare, 1968; Lin et al., 1992). Therefore, it is expected that the presence of peroxide in a biofilm could contribute thermodynamically to ennoblement. Under acidic conditions:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \qquad \emptyset = 1.229 \text{ V SHE} \quad (7)$$

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \qquad \emptyset = 1.776 \text{ V SHE} \quad (8)$$

Extensive calculations were made for the potential contribution by peroxide under the cyclic voltammetry simulation conditions. The Nernst equation gives a thermodynamic potential for reaction (7) as 0.794 V SCE for a pH of 2.9 and a dissolved oxygen concentration of 0.5 ppm (15 mM). Under the same conditions with a peroxide concentration of 2.4 mM, the potential of reaction (8) is 1.289 V SCE. Thus the presence of peroxide could contribute a thermodynamic maximum of 0.495 V to the ennoblement of the OCP under simulated biofilm conditions. The actual contribution would, of course, be less than the thermodynamic value, but this illustrates the relative magnitude of the contribution possible for peroxide under open circuit conditions. Whether or not the peroxide contribution could be significant under corroding conditions where a steady current would be demanded of the cathode reaction would depend on the amount of peroxide that could be produced within the biofilm and the kinetics of the reaction.

The cyclic voltammograms for bare platinum electrodes (Figures 1.3, 1.4, 1.5 and 1.6) indicated the relative positions of peroxide and oxygen peaks. These voltammograms also showed that the cyclic voltammogram from a biofilmed sample could be closely reproduced in these tests only when the proper combinations of pH and peroxide were used. The reduction of oxygen and the oxidation of peroxide observed in the voltammograms were used only as a diagnostic criteria for the identification of oxygen and peroxide in biofilms.

The difference in shapes of voltammograms from samples with and without biofilms (Figures 1.3 and 1.6 at 50 mV/sec) can be explained by the physical effects of the biofilm. The availability of peroxide was high in the biofilm adjacent to the electrode surface, producing a prominent peak corresponding to peroxide oxidation. However, the necessity of diffusing the peroxide formed in the oxic layers of the biofilm to the metal substratum would cause a delay in initiation of the peak. Reductions of about 50% in the diffusion coefficients of inorganic solutes (Aller, 1983) within biofilms as compared to their values in pure water would add to the delay. In contrast, diffusion of peroxide to the electrode from the bulk seawater would be faster, resulting in a gradual increase in the current and a broader peak than that for the biofilmed coupon. Therefore the difference in diffusion of peroxide through the film and across the double layer from the bulk liquid could be used as an explanation for difference in the shape of the peroxide peak.

Even though the bulk seawater was deaerated the oxygen peak appeared to be more prominent in the voltammogram under simulated conditions (Figure 1.6) than for a biofilmed coupon (Figures 1.2 and 1.3). This can be explained by the higher solubility of peroxide in water than oxygen. Henry's law constants are 7.1×10^{4} and 1.3×10^{-3} Moles/atmosphere pressure, for hydrogen peroxide and molecular oxygen respectively (Seinfeld, 1986). This means even though the addition of peroxide is small in volume, production of oxygen is higher compared to direct dissolution of oxygen. The kinetics of oxygen dissolution from the atmosphere are slow compared to direct supply of oxygen from peroxide dissociation into water and oxygen, which is explicit from the seven order magnitude difference in the Henry's law constant. Oxygen is also consumed by the biofilm due to bacterial respiratory processes. Thus the concentration of oxygen within the biofilm on a platinum electrode will be lower than the oxygen concentration at the surface of a bare platinum electrode with peroxide in the bulk solution. Hence the simulated voltammogram in Figure 1.6 appears to have a more prominent oxygen peak than that in Figure 1.3.

Results from the abiotic simulation experiments shown in Figures 1.7 and 1.9 verify the synergistic effects of pH and H_2O_2 in creating the ennoblement phenomenon. The level of ennoblement and the positions of the predominant cyclic voltammetry peaks found in the presence of natural biofilms were reproduced only when the proper combination of pH, H_2O_2 and low oxygen were established in bulk seawater. Under natural biofilm conditions, however, only the chemistry immediately adjacent to the metal surface needs to be at the specified combination of pH, H_2O_2 and oxygen.

Addition of peroxide to aerated and deaerated seawaters produced opposite shifts in potential of the oxygen electrode on platinum (Figure 1.8). This was also observed and explained by Hoare (1968). These data show that in presence of peroxide, ennoblement is possible only when the concentration of oxygen is low in a biofilm. Hoare explained the difference in the potentials of oxygen electrode on platinum to be dependent on the oxygen concentration in the presence of peroxide. The results of experiments conducted using various combinations of pH, peroxide and oxygen as shown in Figures 1.7 and 1.9 illustrate the importance of these three parameters for ennoblement. Only under a low oxygen concentration could pH and peroxide combine to produce ennoblements over 400 mV SCE. These data indicate that in general oxygen concentrations must

be low in the biofilm. Oxygen concentration in a biofilm is also expected to be low due to bacterial utilization of oxygen for their metabolism (Lewandowski et al, 1988). Bacterial utilization of oxygen would produce some concentration of peroxide. Therefore the fall in oxygen concentration could be taken as a first approximation of the rate at which peroxide is produced in a biofilm.

There is an upper limit to the peroxide concentration in a biofilm because enzymes are there to breakdown peroxide; otherwise it would be lethal for the organisms in the film. Christensen et al., (1990) while studying the effect of peroxide in biofilm removal observed that even freshly harvested cells (pure culture in a medium) were inhibited by about 5 mM concentration of peroxide, but biofilm removal did not occur even at 15 mM concentration of peroxide. Biofilms of either *Pseudomonas sp.* NCMB 2021 or *Pseudomonas atlantica* ATCC 19262 both displayed a progressive reduction in sensitivity to hydrogen peroxide upon repeated exposure to this reagent, which was attributed to the biofilm maturation effect, making them able to resist higher peroxide concentration (1990). The concentrations reported in this paper are an order of magnitude less than what was reported for biofilm removal by Christensen et al., (1990).

Cathodic polarization curves for biofilmed platinum had two Tafel slopes implying two electrode reactions instead of the one observed for bare platinum (Figure 1.10). Similar observations were made by Matoda et al.(1990) and Buchanan and Stansbury (1990) using biofilmed stainless steel. Motoda et al.(1990) observed a higher number of diatoms in the biofilms. Diatoms are known to produce peroxide (Palenik et al., 1987; Van Baalen, 1965; Stevens et al., 1973), however Motoda et al., did not measure peroxide in their biofilms. Matoda et al., (1990) did not explain the reason for two Tafel slopes, whereas Buchanan and Stansbury (1990) used depletion of protons as the reason for the appearance of two Tafel slopes. Having identified and measured peroxide in a biofilm, and knowing that both oxygen and peroxide reduction deplete protons, it is hypothesized that the first Tafel slope is due to reduction of peroxide and the second slope is due to reduction of oxygen. Since the concentration of peroxide is limited in the biofilm, depletion of peroxide leads to the fall in the curve which then coincides with oxygen reduction on the bare platinum.

The data presented in Figure 1.11 showed that peroxide shifted the cathodic polarization curve for platinum towards a higher current density range. This confirmed the increase in cathodic kinetics as a result of the presence of peroxide in biofilms. The Tafel slopes were 0.12 V/decade of current density. This value is almost same for both peroxide and oxygen in natural seawater. However, there was a two order of magnitude increase in both the exchange current density and limiting current density for peroxide over that for oxygen. This showed that the presence of peroxide in a biofilm would contribute to ennoblement.

The rate of OCP decay for ennobled electrodes were almost same (Figure 1.13) in presence of active and inactive catalase enzyme additions. Figure 1.14 showed a rapid decrease in dissolved oxygen of the solution roughly coinciding with the active-inactive transition of the enzyme and formation of the surface film. It is thought that elimination of oxygen from the solution was brought about by a combination of blocking dissolution of oxygen from the atmosphere by the inactive enzyme film and scavenging of oxygen from solution by the biofilm. The loss of dissolved oxygen as well as peroxide contributed to the rapid decay of OCP values. This leads to the important conclusion that at least some dissolved oxygen in the bulk seawater is necessary for ennoblement. Once oxygen is eliminated from the system, peroxide production should also cease, and the synergistic effects of peroxide and pH will be lost.

Our chemical simulation experiments indicated that production of peroxide and lowering of pH can be primary contributors to ennoblement. The decrease in pH under microcolonies of organisms from our biofilms grown on marine agar indicate the possibility of low pH in the biofilm. this is supported by the results from our cyclic voltammetry simulation studies. This is consistent with the idea that the pH under a mature biofilm can be acidic, and that it can contribute to ennoblement in cooperation with the peroxide that is produced in the biofilm.

Summary And Conclusions

The phenomenon of ennoblement of passive alloys in the presence of marine biofilms has been studied using the oxygen electrode on platinum as a model system. Lowering in pH and production of hydrogen peroxide within biofilms have been shown to be primary contributors to ennoblement. Contribution of peroxide to ennoblement arises from its relatively noble thermodynamic potential at low pH. It is speculated that a secondary contribution could arise through the peroxide either directly or indirectly as a contributor to oxidation of reduced metabolites in a biofilm (such as sulfides to elemental sulfur or sulfuric acid) thereby lowering the pH. Specific conclusions from this work are:

1. H_2O_2 was found in measurable quantity in the biofilms at both test locations.
2. Under our test conditions the observed amount of ennoblement required a combination of low oxygen and acidic pH with low millimolar concentrations of peroxide.
3. The presence of dissolved oxygen in the bulk solution is required for ennoblement.

Acknowledgments

This work was sponsored by the Office of Naval Research under contract number 01490J1947. The authors would like to thank Prof. Dr. G.V. Subba Rao, Director, Central Electrochemical Research Institute for allowing the former author P.C to use his laboratory facility at OPMEC during his visit to India late November, 1992. M. Eashwar, Scientist at OPMEC, Tuticorin is acknowledged for the ennoblement data on platinum. Professors D.L. Kirchman of College of Marine Studies and Professor. M. Fletcher of Center of Marine Biotechnology, Baltimore are acknowledged for their constant support and directions in conducting this work.

References

Aller R C (1983) The Importance of the Diffusive Permeability of Animal Burrow Linings in Determining Marine Sediment Chemistry, J Marine res., 41: 299-322.

Anson F C (1983) Adventures in Electrocatalysis, David C Grahame award address of the physical electrochem Div.

Anson F C, Ni C L, Saveant J M (1985) Electrocatalysis at Redox Polymer Electrodes with Separation of the Catalytic and Charge Propagation Roles. Reduction of O2 to H2O2 as Catalyzed by Cobalt (II) Tetrakis (4-N-methylpyridyl) Porphyrin, J Amer Chem Soc, C Vol. 107, 12, p. 3442.

Bettelheim A, Kuwana T (1979) Rotating-ring-disc Analysis of Iron Tetra (N-methyl pyridyl) Porphyrin in Electrocatalysis of Oxygen, Analytical Chemistry, 51, 13, 2257- 2260.

Bettelheim A, Chan R J H, Kuwana T (1979) J Electroanal Chem, 99: 391.

Bettelheim A, Chan R J H, Kuwana T (1980) Electroanalysis of Oxygen Reduction Part III. Selective Reduction to Hydrogen Peroxide or Water Using Polymeric Attachment of Metalloporphyrins, *J Electroanal Chem*, 110: 93-102.

Bettelheim A, White B A, Murray R C (1987) Electrocatalysis of Dioxygen Reduction in Aqueous Acid and Base by Multimolecular Layer Films of Electropolymerized Cobalt Tetra (O-aminophenyl) Porphyrin, J Electroanal Chem. 217: 271-286.

Britton L N (1985) Feasibility Studies on the use of Hydrogen Peroxide to Enhance Microbial Degradation of Gasoline, Texas Research Institute, American Petroleum Institute, Report 36, Washington D.C.

Brock T D, Madigan M T (1988) Biology of Microorganisms, Fifth Edition, Prentice Hall, Englewood Cliffs, NJ 07632, pp. 1-788.

Buchanan R A, Li P, Zhang X, Dowling N D E, Stansbury E E, Danko J C, White D C (1989) Electrochemical Field Studies of Fresh Water Microbiologically Influenced Corrosion, A progress report to the EPRI.

Buchanan R A, Stansbury E E (1990) Microbially Induced Corrosion and Biodeterioration, In: Dowling N J, Mittelman M W, Danko J C (eds) Proc International Congress on Microbially Induced Corrosion, The Univ. of Tennessee, Knoxville.

Center for Biofilm Engineering News (1994) Montana State University, Bozeman, Montana, Vol. 2, No. 1.

Chan R J H, Su Y O, Kuwana T (1985) Electrocatalysis of Oxygen Reduction 5. Oxygen to Hydrogen Peroxide Conversion by Cobalt (II) Tetrakis (N-methyl-4 pyridyl) Porphyrin, Inorg. Chem, 24: 3777-3784.

Chandrasekaran P, Dexter S C (1993a) Mechanism of Potential Ennoblement on Passive Alloys due to marine biofilms, Proc Corrosion '93, Paper No. 493, NACE, Houston, TX.

Chandrasekaran P, Dexter S C (1993b) Factors Contributing to the Ennoblement of Stainless Alloys by Seawater Biofilms, Proc 12th International Corrosion Congress, held Sept 19-24, 1993, (Houston, TX: NACE, 1993), Vol. 5B, pp. 3696-3707.

Chandrasekaran P, Dexter S C (1994) Bacterial Metabolism in Biofilm Consortia: Consequences for Potential Ennoblement, Proc Corrosion '94, Paper No. 276, NACE, Houston, TX.

Chandrasekaran P (1995) Mechanism of Potential Ennoblement on Passive Metals Due to Biofilms in Seawater, Ph D dissertation, College of Marine Studies, University of Delaware, Newark, DE.

Chandrasekaran P, Dexter S C (1995) Measurement of Acid pH Within Marine Biofilms on Passive Metals (Communicated to Biofouling).

Characklis W G, Wilderer P A (eds) (1989) Structure and Function of Biofilms, A report of the Dahlem Workshop on Structure and Function of Biofilms, held Nov 27-Dec 2, 1988, A Wiley-Interscience Publication, John-Wiley and Science, New York, pp.129-190.

Christensen B E, TrØnnes H N, Vollan K, SmidsrØd O, Bakke R (1990) Biofilm Removal by Low Concentrations of Hydrogen Peroxide, Biofouling, Vol. 2, No. 2, pp. 165-175.

Collman J P, Anson F C, Bencosme S, Chong A, Collins T, Denisevich P, Evitt E, Geiger T, Ibers J A, Jameson G, Konai Y, Koval C, Meier K, Oakley P, Pettman R, Schmittou E, Sessler J (1981) Molecular Engineering: The Design and Synthesis of Catalysts for the Rapid 4-electron Reduction of Molecular Oxygen to Water, In: Trost B M, Hutchinson C R (eds) Organic Synthesis Today and Tomorrow, Pergamon, New York, p. 29-45.

Cooper W J, Zika R G (1983) Photochemical Formation of Hydrogen Peroxide in Surface and Ground Waters Exposed to Sunlight, Science, 220:711-712.

Cooper W J, Zika R G, Pestasne R G, Plane J M C (1988) Photochemical Formation of H2O2 in Natural Waters Exposed to Sunlight, Environmental Science and Technology 22:1156- 1160.

Corpe W (1970) Attachment of Marine Bacteria to Solid Surfaces, Chapter 4, In: Manly R S (ed) Adhesion in Biological Systems, Academic Press, New York, pp. 73-87.

Costilow R N (1981) Biophysical Factors in Growth, Chapter 6, In: Gerhardt P (ed) Manual of Methods for General Bacteriology, American Society for Microbiology, Washington D.C., p. 66.

Dexter S C, Gao G Y (1988) Effect of Seawater Biofilms on Corrosion Potential and Oxygen Reduction of Stainless Steel, Corrosion, 44: p. 717.

Dexter S C, Lin S H (1988) Mechanism of Corrosion Potential Ennoblement by Marine Biofilms, Proc 7th Intl Cong on Marine Corrosion and Fouling, Valencia, Spain.

Dexter S C, Zhang H J (1990), Effect of Biofilms on Corrosion Potential of Stainless Alloys in Estuarine Waters, Proc 11th Intl Corrosion Congress, Florence, Italy, 4: 333-340.

Dexter S C, Zhang H J (1991) Effect of Biofilms, Sunlight, and Salinity on Corrosion Potential and Corrosion Initiation of Stainless Alloys, EPRI Final report NP-7275, Research Project 2939-4.

Dexter S C, Chandrasekaran P, Zhang H J, Wood S (1993) Microbial Corrosion in Marine Environments: Effect of Microfouling Organisms on Corrosion of Passive Metals, In: Videla H, Lewandowski Z, Lutey R (eds), Proc 2nd U S A / Argentina workshop on Biocorrosion and Biofouling, Buckman Laboratories, Memphis, TN, (1993), p. 171.

Eashwar M, Maruthamuthu S, Sathiyanarayanan S, Balakrishnan K (1993) Ennoblement of Stainless Alloys by Marine Biofilms: an Alternative Mechanism, In: Proc 12th Intl Corrosion Congr. NACE International, Houston, TX, pp. 3708-3716.

Forshey P A, Kuwana T (1983) Electrochemistry of Oxygen Reduction 4. Oxygen to Water Conversion by Iron (II) Tetrakis (N-Methyl-4-Pyridyl) Porphyrin via Hydrogen Peroxide, Inorganic Chem, 22, 5, pp. 699-707.

Fridovich I (1974) Chapter 11, In: Hayaishi (ed), Molecular Mechanisms of Oxygen Activation, Academic Press, New York, pp. 453-477.

Ghiorse W C (1988) Microbial Reduction of Manganese and Iron, Chapter 6, In: Zehnder A J B (ed) Biology of Anaerobic Microorganisms, Wiley Series in Ecological and Applied Microbiology, pp. 313-316.

Gottschalk G (1986) Bacterial Metabolism, Second Edition, Springer-Verlag, p. 1-341.

Haag W R, Hoigne J (1986) Singlet Oxygen in Surface Waters. 3. Photochemical Formation and Steady-State Conc. in Various Types of Waters, Environmental Science and Technology, 20: 341-348.

Hancock R D, Martell A E (1989) Ligand Design for Selective Complexation of Metal ions in Aqueous Solution, Chem Reviews, 89: 1875-1914.

Hoare J P (1968) In: The Electrochemistry of Oxygen, Interscience Publishers, A Div. of John Wiley & Sons, New York.

Johnsen R, Bardal E (1985) Cathodic Properties of Different Stainless steels in Natural Seawater, Corrosion, 41: 296-302.

Johnsen R, Bardal E, (1986) The Effect of Microbial Slime Layer on Stainless Steel in Natural Seawater, Proc of Corrosion 86, Paper No.227, NACE, Houston, TX.

Kobayashi N, Nishiyama Y (1984) Catalytic Electroreduction of Molecular Oxygen at Glassy Carbon Electrodes with Immobilized Iron Porphyrins, J Electroanal Chem, 181: 107-117.

Kuwana T, Fujihara M, Sunakawa K, Osa T (1978) Catalytic Electroreduction of Molecular Oxygen Using Water Soluble Iron Porphyrin, J Electroanal Chem, 88: 299-303.

Lewandowski Z, Lee W C, Characklis W G, Little B J (1988) Dissolved Oxygen and pH Microelectrode Measurements at Water Immersed Metal Surfaces, Proc Corrosion '88, Paper No. 93, NACE, Houston, TX.

Lewandowski Z, Funk T, Roe F, Little B J (1994) Spatial Distribution of pH at Mild Steel Surfaces Using an Iridium oxide Microelectrode, In: Kearns J R, Little B J (eds) Microbiologically Influenced Corrosion Testing, ASTM STP 1232, American Society for Testing and Materials, Philadelphia, p. 61-69.

Lin C C, Smith F R, Ichikawa N, Itow M (1992) Electrochemical Potential Measurements Under Simulated BWR Water Chemistry Conditions, Corrosion, 48: 16-28.

Lock M A, Wallace R R, Costerton J W, Ventullo R M, Charlton S E (1984) River Epilithon: Toward a Structural-Functional Model, Oikos, Copenhagen 42: 10-22.

Luther G W III (1990) Chapter 6 in: Stumm W (Ed) Aquatic Chemical Kinetics Reaction Rates of Processes in Natural Waters, Wiley Interscience, p. 173.

Mansfeld F, Little B J (1991) A Technical Review of Electrochemical Techniques Applied to Microbiologically Influenced Corrosion, Corrosion Science, 32: 247-272.

Mansfeld F, Tsai R, Shih H, Little B J, Ray R, Wagner P (1992), An Electrochemical and Surface Analytical Study of Stainless Steels and Titanium Exposed to Natural Seawater, Corrosion Science, 33: 445-456.

Maruthamuthu S, Eashwar M, Raja S S, Balakrishnan K (1993) Effects of Microfouling and Light/Dark Regimes on Corrosion Potentials of two Stainless Alloys in Seawater, Biofouling, 7: 257-265.

Millero F J, LeFerriere A, Fernandez M, Hubinger S, Hershey J P (1989) Oxidation of H2S with H2O2 in Natural Waters, Environmental Science and Technology, 23: 209-213.

Mitchell R L, Anderson I C (1965) Catalase Photoinactivation, Science, 150: 74.

Mollica A, Trevis A (1976) Influence of the Microbiological Film on Stainless Steels in Natural Seawater, Proc 4th Intl Cong on Marine Corrosion and Fouling, (Juan-Les-Pins, Antibes, France) p. 351.

Mollica A, Ventura G, Traverso E, Scotto V, (1988) Cathodic behaviour of Nickel and Titanium in Natural Seawater, International Biodeterioration, 24: 221-230.

Mollica A (1992) Biofilm and Corrosion on Active-Passive alloys in Seawater, International Biodeterioration and Biodegradation, 29: 213-229.

Morris J G (1984) Chapter 3, In: Codd G A, Aspects of Microbial Metabolism and Ecology, Published for the Soc. for General Microbiology, Special Publication of the Society for General Microbiology, Academic Press, 11, p. 59.

Motoda S, Suzuki Y, Shinohara T, Tsujikawa S (1990) The Effect of Marine Fouling on the Ennoblement of Electrode Potential for Stainless Alloys in Seawater, Corrosion Science, 31: 515-520.

Ozer D, Harth R, Mor U, Bettelheim A (1989) Electrochemistry of Various Substituted Aminophenyl Iron Porphyrins. Part II. Catalytic Reduction of Dioxygen by Electropolymerized Films, J Electroanal Chem, 266: 109-123.

Palenik B, Zafiriou O C, Morel F M M (1987) Hydrogen Peroxide Production by a Marine Phytoplankter, Limnology and Oceanography, 32 (6): 1365-1369.

Palenik B, Morel F M M (1988) Dark Production of H2O2 in the Sargasso Sea, Limnol. Oceanogr 33 (6, part 2), pp. 1606-1611.

Pourbaix M (1974) Atlas of Electrochemical Equilibria in Aqueous Solutions,NACE, Houston, TX, USA, pp. 97-111.

Price N M, Morel F M M (1990) Chapter. 8, In : Stumm W (ed) Aquatic Chemical Kinetics Reaction Rates of Processes in Natural Waters, Wiley Interscience, p. 235.

Salvago G, Fumagalli G, Taccani G, Cristiani P, Rocchini G (1992), Electrochemical and corrosion behaviour of passive and fouled metallic materials in seawater, In: Sequeira C A C, Tiller A K (eds) Microbial Corrosion, Proceedings of the 2nd EFC workshop Portugal, 1991, Published for the European Federation of Corrosion by the Institute of Materials.

Saveant J M, Vianello E (1963) Electrochim Acta, 8: 905.

Scotto V, DiCintio R, Mercenaro G (1985) The Influence of Marine Aerobic Microbial Film on Stainless Steel Corrosion Behaviour, Corrosion Science, 25: 185-194.

Scotto V (1988) Electrochemical Studies of Biocorrosion of Stainless Steel in Seawater, Proc EPRI Workshop on Microbial Induced Corrosion, Electric Power Research Inst., PaloAlto, CA, p.A-1.

Seinfeld J (1986) In: Atmospheric Chemistry and Physics of Air Pollution, John Wiley and Sons,Inc., New York.

Stevens S E Jr, Patterson C O P, Myers J (1973) The Production of Hydrogen Peroxide by Blue- Green Algae, J Phycol, 9: 427-430.

Tarasevich M R, Radiyschkina K A, Androuseva S I (1977) Electrocatalysis of Oxygen Reduction on Organic Metallic Complexes, Bioelectrochemistry and Bioenergetics, 4: 18-29.

Therien M J, Bowler B E, Selman M A, Gray H B, Chang I J, Winkler J R (1991) Long-Range Electron Transfer in Heme Proteins, Porphyrin-Ruthenium Electronic Couplings in Three Ru(His) Cytochromes C, Chapter 12, In: Bolton J R, Mataga N, McLendon G (eds) Electron Transfer in inorganic organic and biological systems, Advances in CSC Symp.Series 2, Chemistry Series 228, American Chemical Society, Washington D.C.

Van Baalen C (1965) Quantitative Surface Plating of Coccoid Blue-Green Algae, J Phycol 1:19- 22.
Van den Brink F, Barendrecht E, Visscher W (1980) The Cathodic Reduction of Oxygen, A Review with Emphasis on Macrocyclic Organic Metal Complexes as Electrocatalysts, Recueil, Journal of the Royal Netherlands Chemical Society, 99, 9, p. 253.
Vasudevan P, Santosh, Mann N, Tyagi S (1990) Transition Metal Complexes of Porphyrins and Phthalocyanins as Electrocatalysis for Dioxygen Reduction, Transition Metal Chemistry, 15: 81-90.
Washburn E W (1928) Ed-in-Chief, International Critical Tables of Numerical Data, Physics, Chemistry and Technology, Vol. III, International Research Council and The National Academy of Sciences, McGraw Hill Book Company, Inc. NY, (1928), p. 54.
Worthington Manual, Enzymes related biochemicals, Worthington biochemical corporation, Freehold, NJ, U. S. A. 07728.
Yeager E (1976) Mechanisms of Electrochemical Reactions on Non-Metallic Surfaces, In: Electrocatalysis on Non-Metallic Surfaces, NBS Spec Publ 455, Gaithersburg, MD, pp. 203-219.
Yeager E (1986) Dioxygen Electrocatalysis: Mechanisms in Relation to Catalyst Structure, The 1985 Berzelius Lecture, J of Molecular catalysis, 38: 5-25.
Zafiriou O C, Dubien J J, Zepp R G, Zika R G (1984) Photochemistry of Natural Waters, Environmental Science and Technology, Vol 18, No 12, pp 358A-371A.
Zepp R G, Wolfe N L, Baughman G L, Hollis R C (1977) Singlet Oxygen in Natural Waters, Nature (London), 267: 421-423.
Zepp R G, Schlotzhauer P F, Sink R M (1985) Photosensitized Transformations Involving Electronic Energy Transfer in Natural Waters:Role of Humic Substances, Environmental Science and Technology, 19: 74-81.
Zika R G, Moffett J W, Petasne R G, Cooper W J, Saltzman E S (1985) Geochemica et Cosmochimica Acta, 49: 1173-1185.

Table 1.1 Observed pH of metabolites of colonies grown on marine agar from microorganisms scraped from biofilms.

pH indicating dye in the agar	**Total number of colonies**	**Number of colonies in each pH range.**	**% showing acid pH.**
Phenol red below 6.8 yellow above 8.2 red	37	26 Yellow Zero red Remainder white	70.27
Chlorophenol red below 4.8 yellow above 6.4 red	14	5 Yellow Remainder white	35.71
Bromocresol green below 3.8 yellow above 5.4 blue	32	12 Yellow Remainder bluish white	37.5
Bromophenol blue below 3.0 yellow above 4.6 blue	28	10 Yellow Remainder bluish white	35.71
None	31	6 Yellow Remainder white	Not applicable.

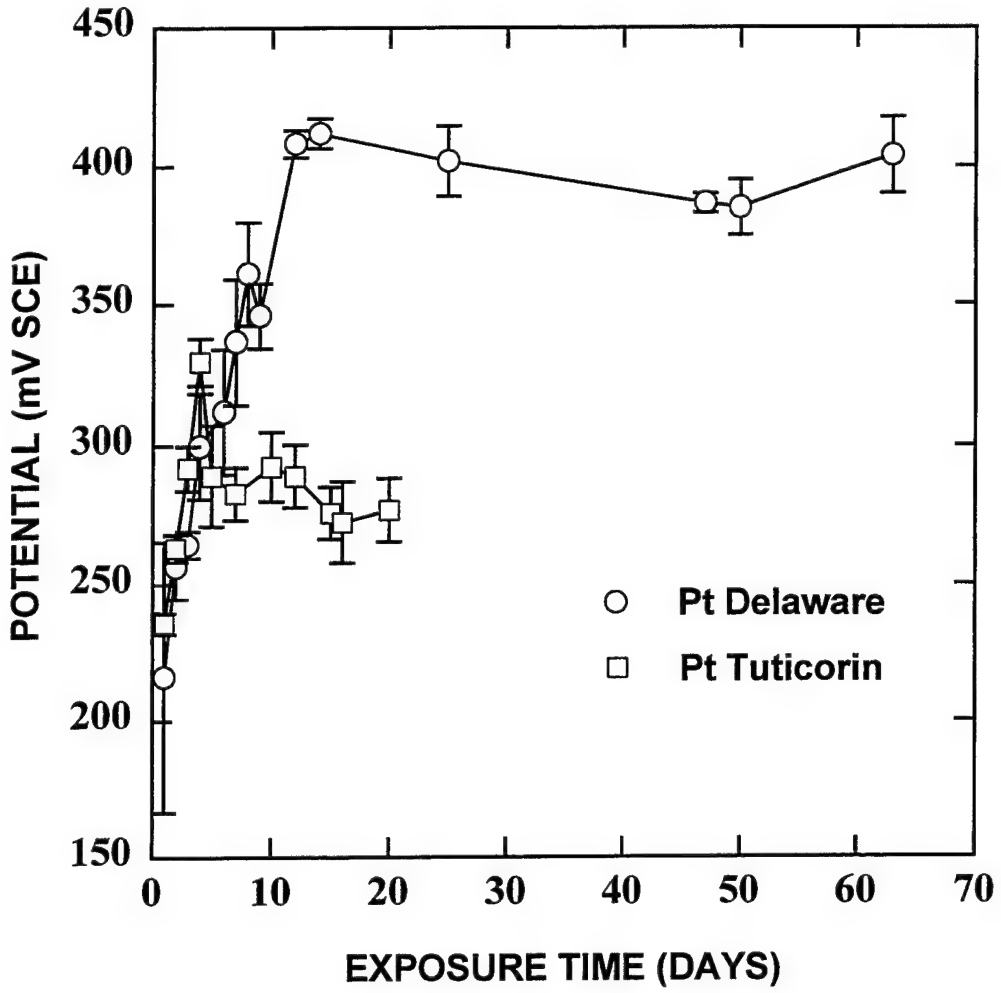

Figure 1.1 Open circuit potential of platinum in Lower Delaware Bay seawater and in the coastal waters of Tuticorin, India.

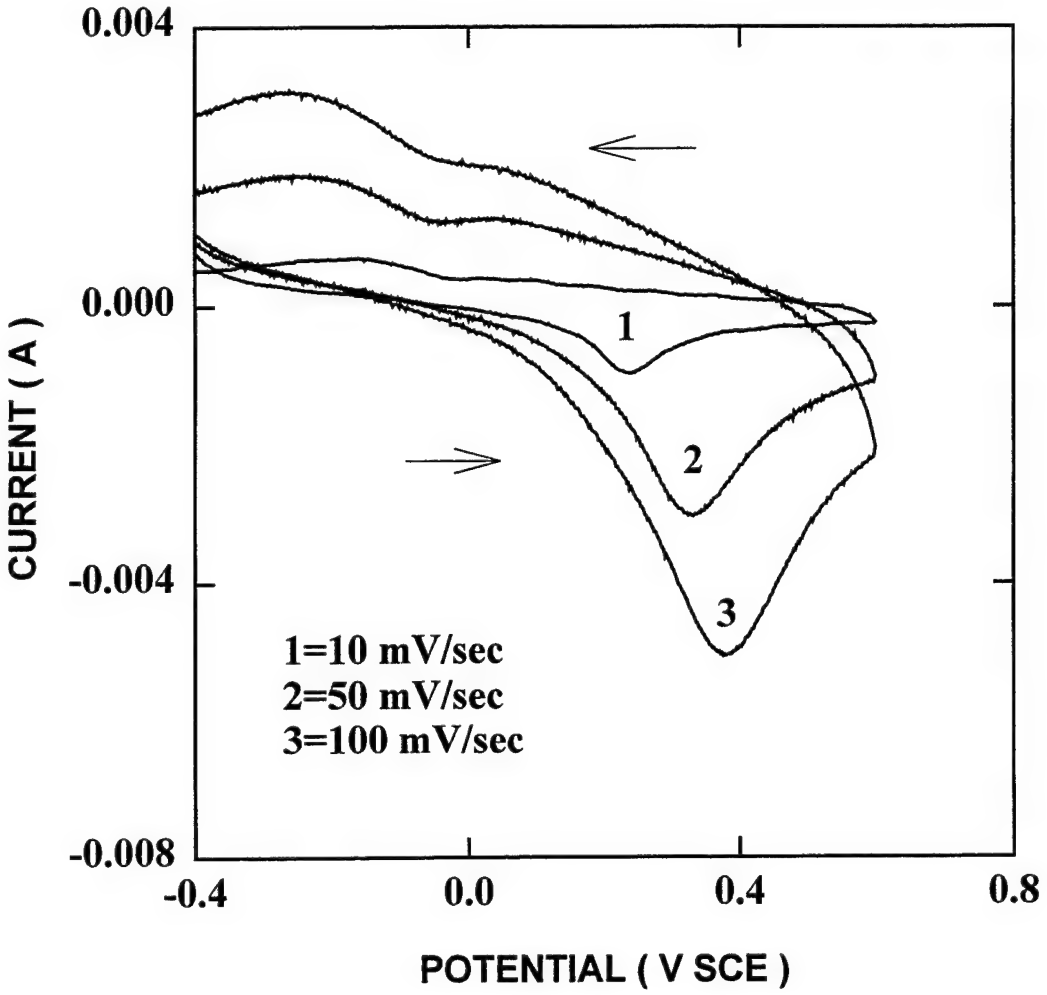

Figure 1.2 Effect of scan rate on the cyclic voltammogram of a biofilmed platinum electrode. The peaks are shifted in the forward direction for voltammograms 2 and 3.

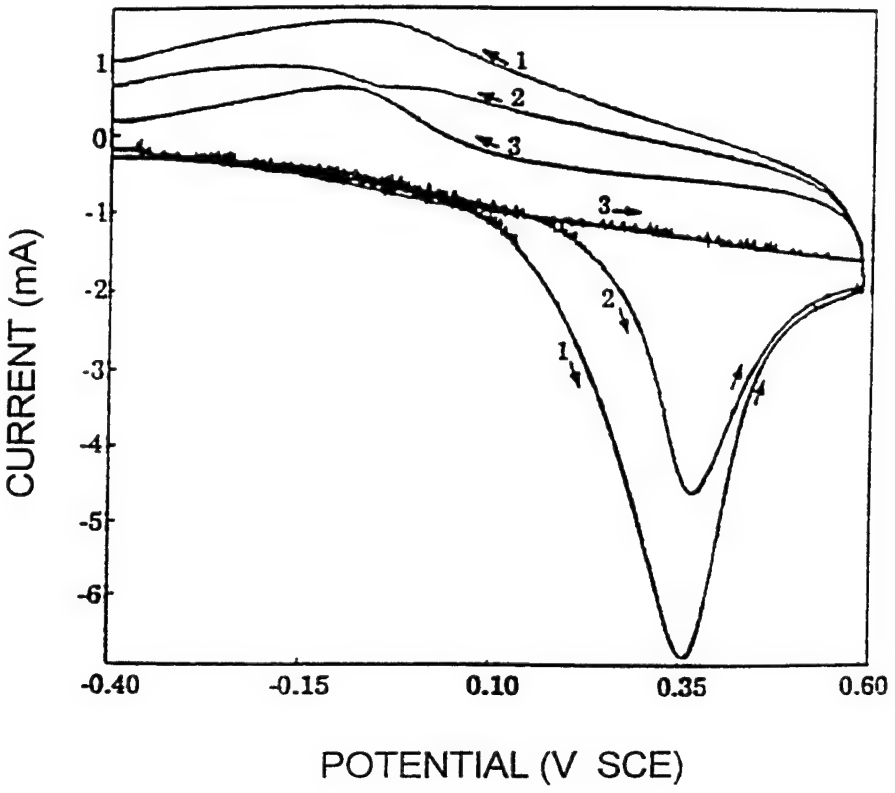

Figure 3 Voltammograms of biofilmed (curves 1 and 2) and bare (curve 3) platinum electrodes in natural seawater (scan rate 50 mV/sec). Curves 1 and 2 represent successive runs on the same electrode.

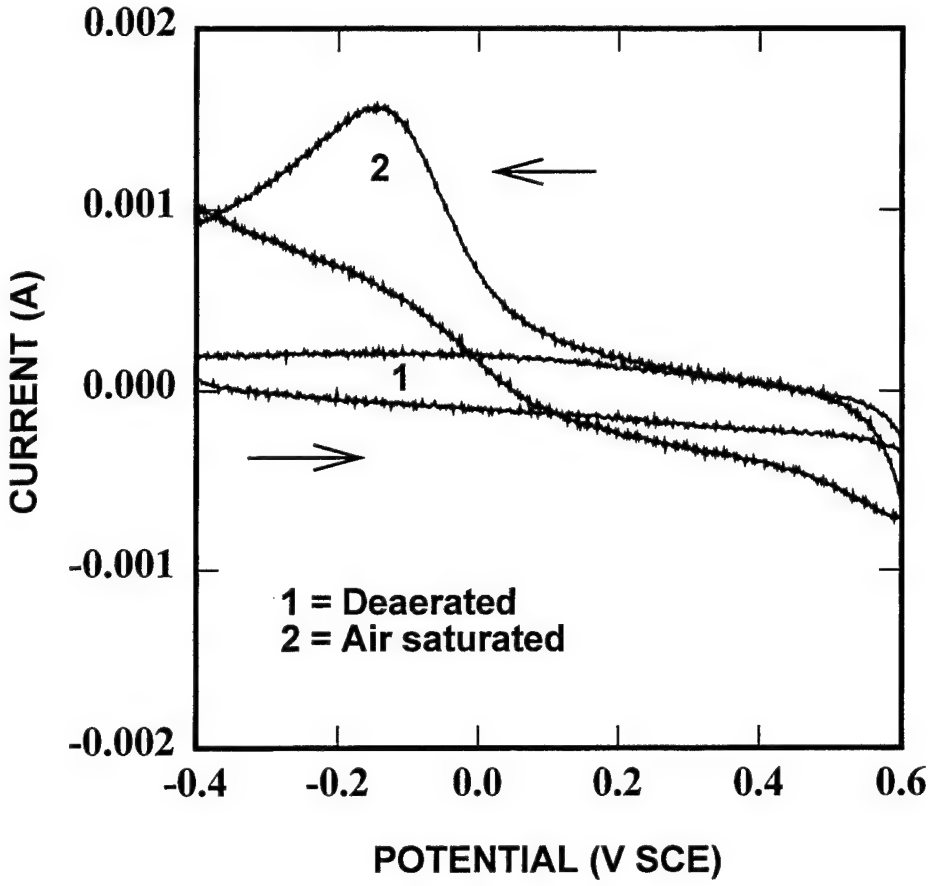

Figure 1.4 Voltammograms of bare platinum electrodes in natural seawater at a scan rate of 50 mV/sec. Curve 1 represents the deaerated condition, while curve two was taken with the oxygen concentration at air saturation.

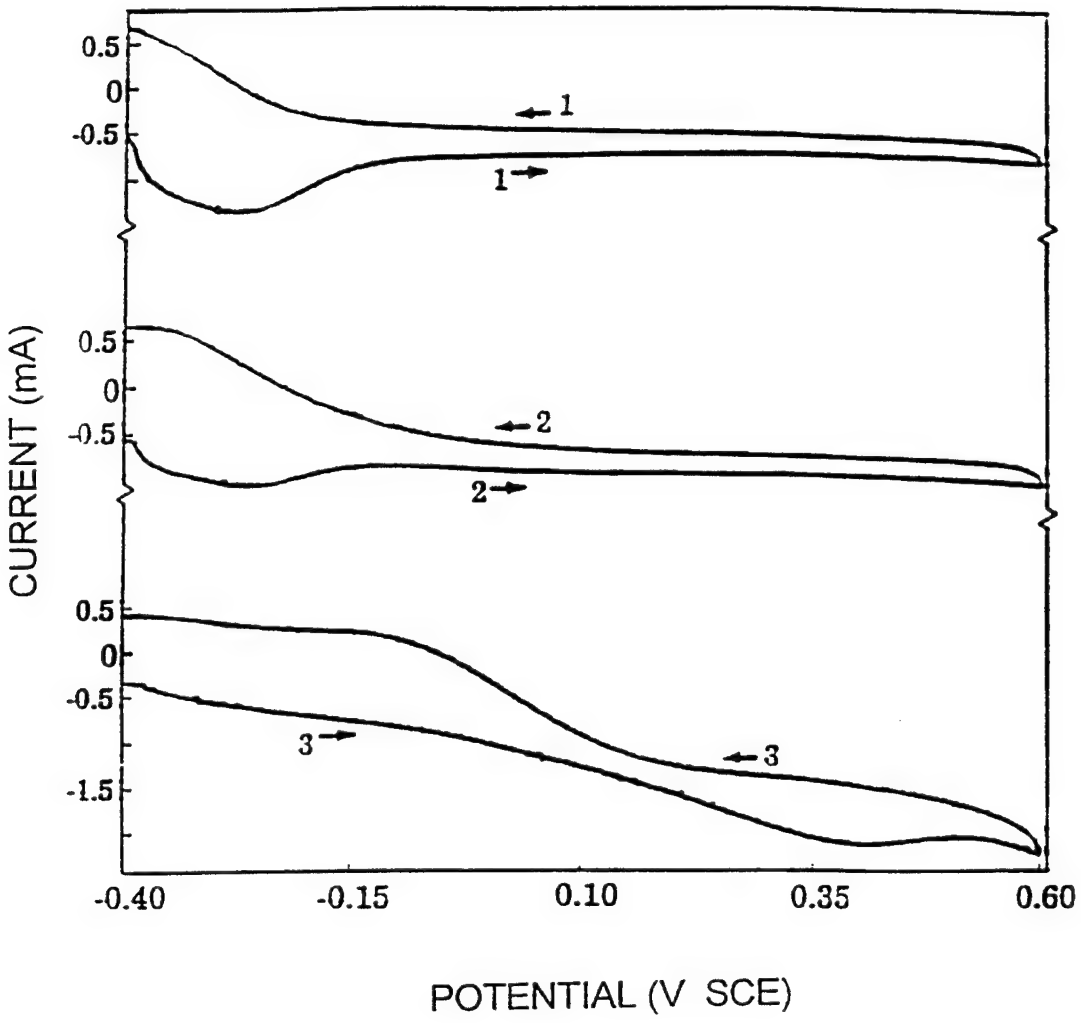

Figure 5 Voltammograms of bare platinum electrodes (scan rate 50 mV/sec) under simulated biofilm chemistry in one liter of seawater. 1) Deaerated at pH 2.93; 2) Same as condition 1 with 0.48 mM peroxide; 3) Same as condition 1 with 1.44 mM peroxide.

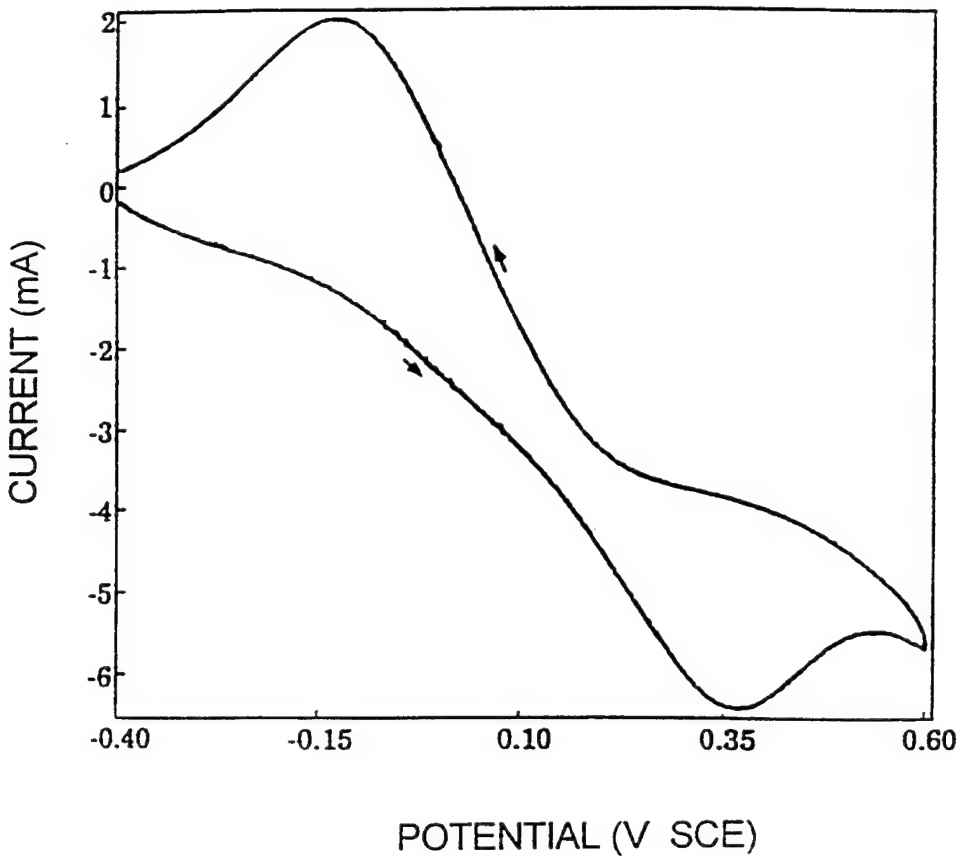

Figure 6 Voltammogram of bare platinum electrode in seawater deaerated to less than 0.5 ppm dissolved oxygen with 2.5 mM peroxide added at pH 2.8 to 2.9.

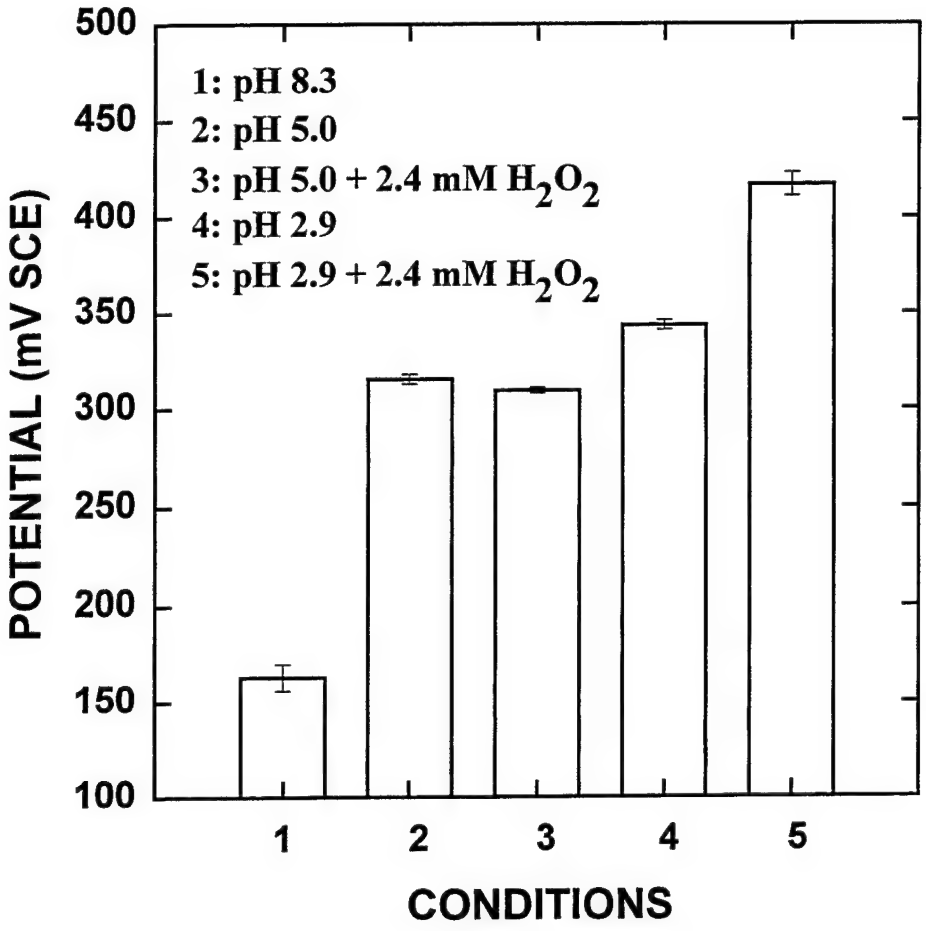

Figure 1.7 OCP of platinum in deaerated (0.55 +/- 0.05 ppm O_2) natural seawater at various pH and peroxide levels.

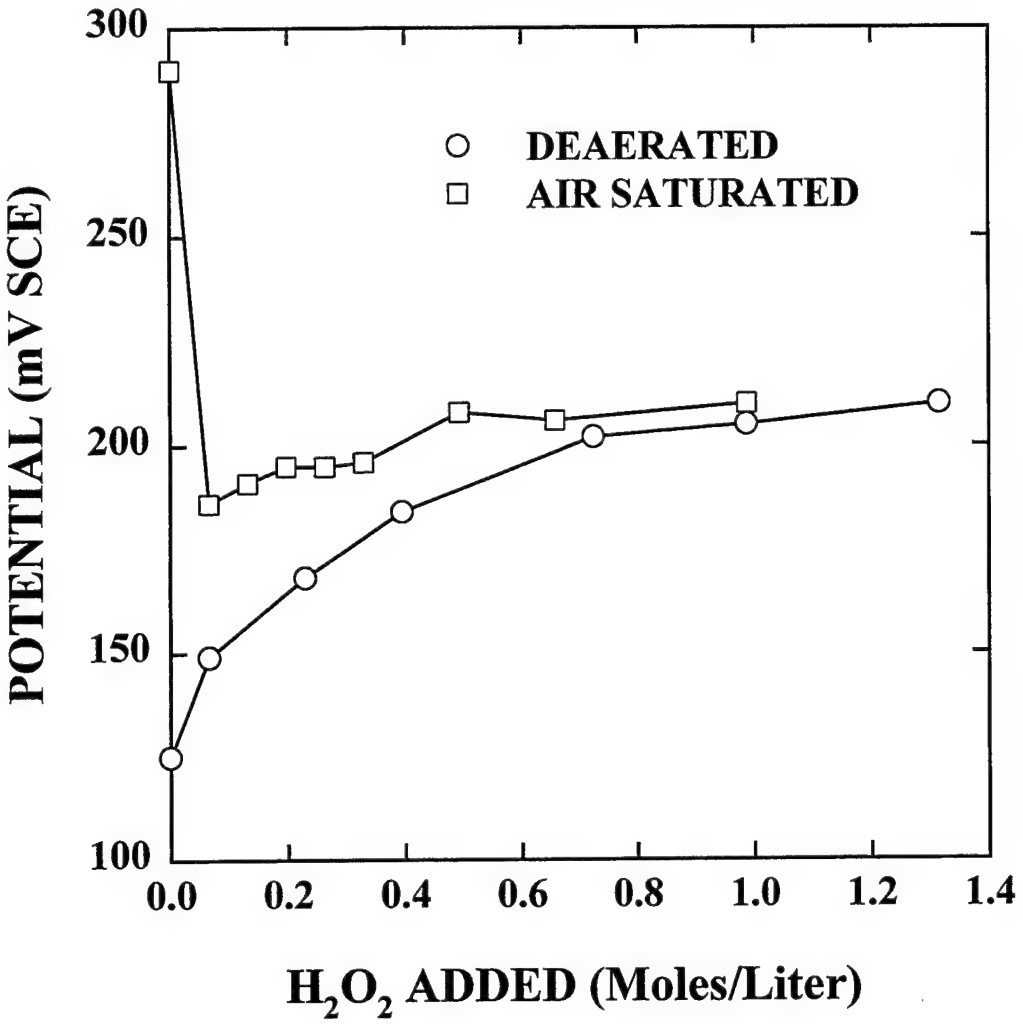

Figure 1.8 Effect of peroxide addition on platinum potential under deaerated (0.2 ppm oxygen) and air saturated seawater conditions at pH 8.3.

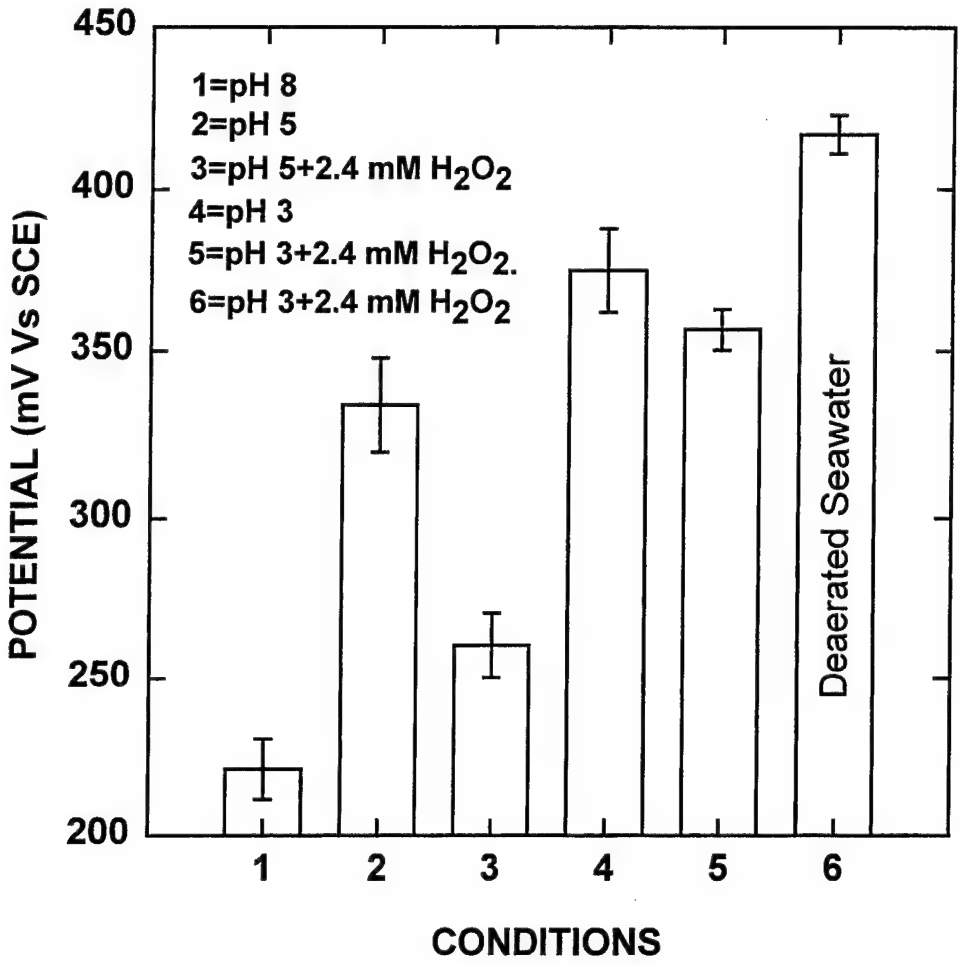

Figure 1.9 OCP of platinum in air saturated natural seawater due to changes in pH and peroxide. For comparison, condition 6 is deaerated the same as condition 5 in Figure 1.7.

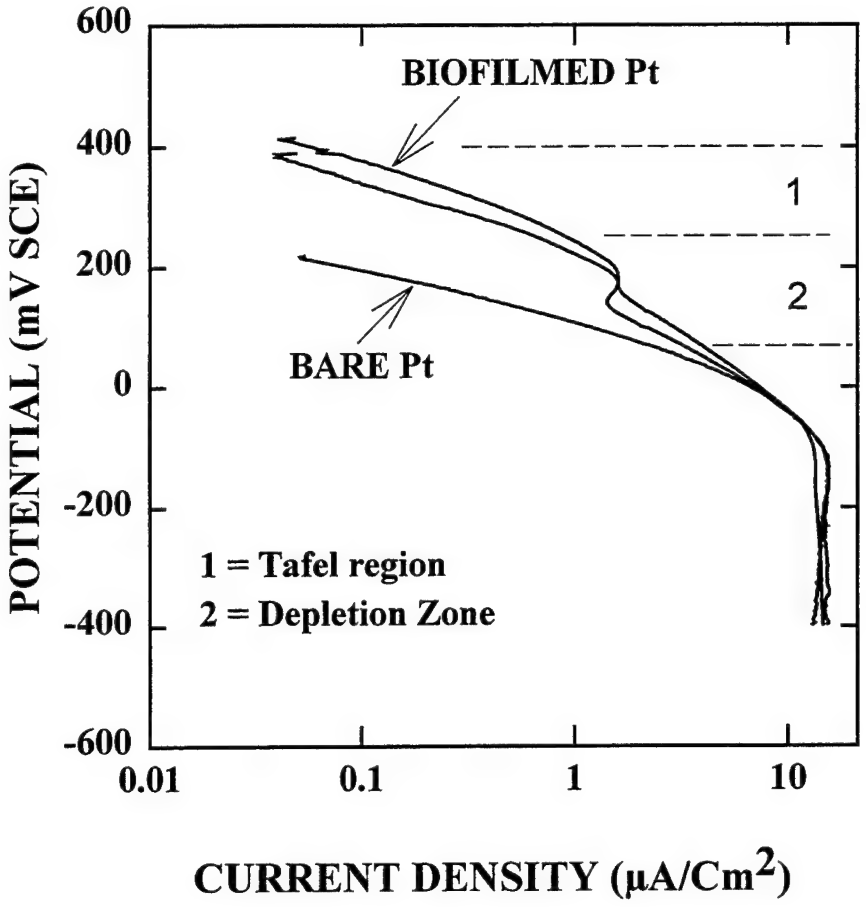

Figure 1.10 Cathodic polarization curves for platinum in natural seawater with and without biofilms.

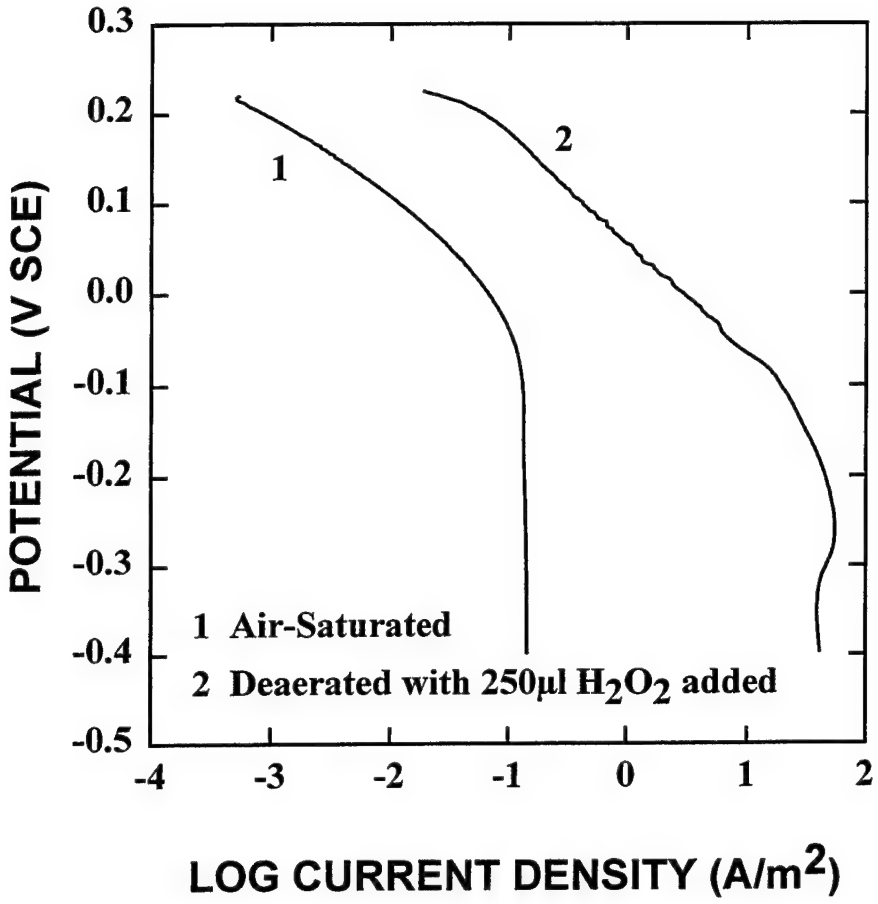

Figure1.11 Effect of oxygen and peroxide on cathodic kinetics of platinum in natural seawater.

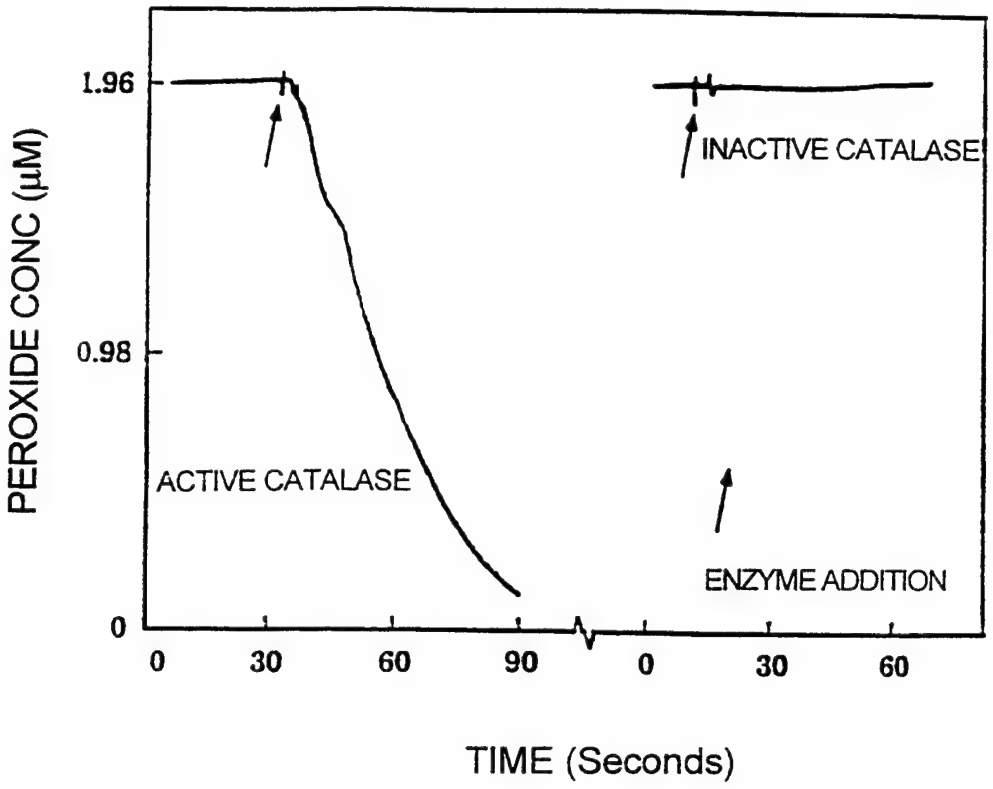

Figure 12 Typical spectrophotometer data showing change in absorbance (or peroxide concentration) upon addition of active or inactive catalase enzyme.

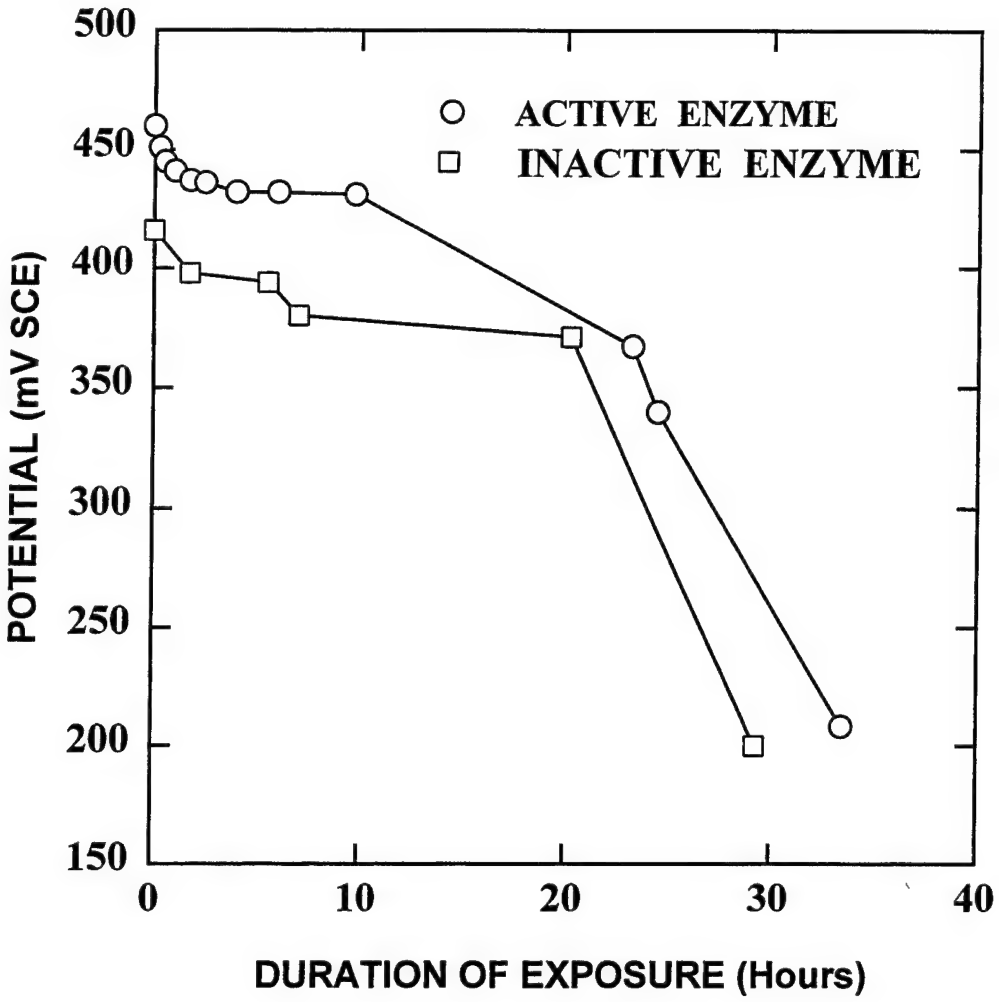

Figure 1.13 Effect of catalase enzyme additions (both active and inactive) on the OCP of an ennobled platinum electrode.

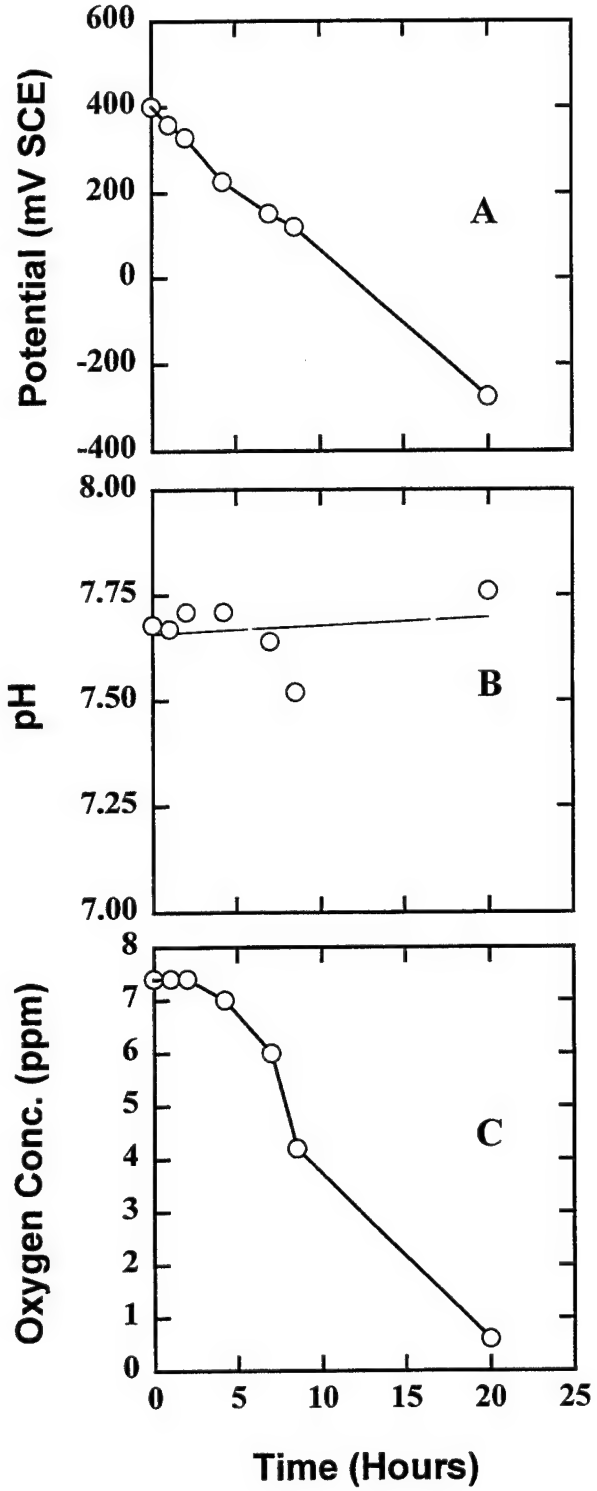

Figure 1.14 Effect over time of adding active catalase to the bulk solution on: A) OCP of an ennobled platinum electrode, B) pH of the bulk solution, and C) dissolved oxygen concentration in the bulk solution.

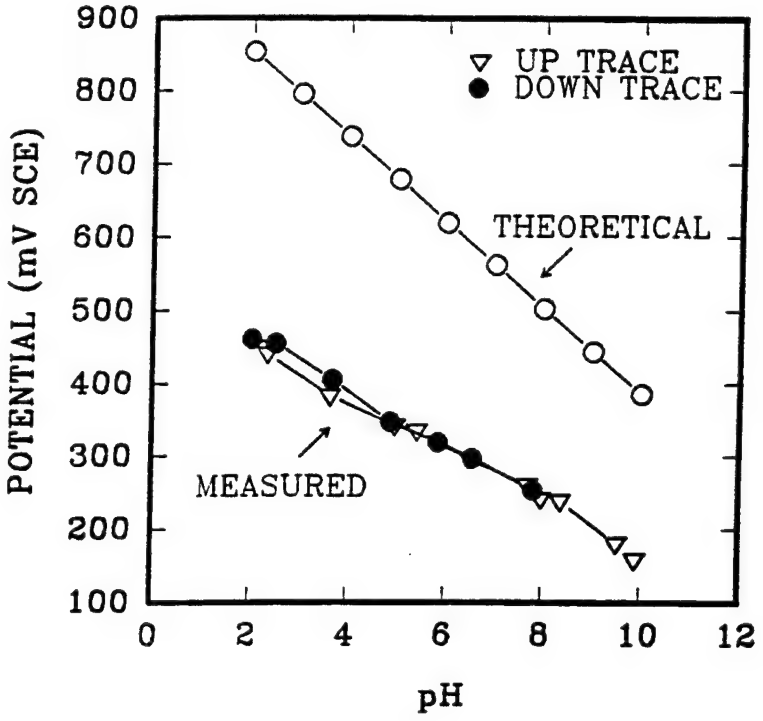

Figure 1.15 Response of oxygen electrode potential on platinum to variations of pH in air saturated Delaware Bay seawater.

Chapter 2

Thermodynamic and Kinetic Factors Influenced by Biofilm Chemistry Prior to Passivity Breakdown

Abstract

Contributions to ennoblement by various factors were studied on platinum and S44660 stainless alloy. Thermodynamics was found to be the major contributor for platinum, whereas cathodic kinetics also contributed to ennoblement on S44660 stainless alloy. Cyclic voltammetry indicated the controlling oxidation-reduction couple for ennoblement involved oxygen in some form (oxygen and peroxide). Passive current density was found to vary little between bare and biofilmed coupons for both S44660 stainless alloy and platinum. This implied that the contribution to ennoblement by passive current density shift is insignificant on platinum and S44660 stainless alloy as used in this study.

Keywords: Ennoblement, thermodynamics, kinetics, exchange-current, Tafel-slope, passive-current, seawater, biofilms, alloy S44660, platinum.

Introduction

Seawater biofilms ennoble the open circuit potentials (OCP) of passive metals and alloys[1]. Biofilms also change the shape of the passive metal cathodic polarization curve as has already been reported by various investigators[1-5]. Such a change in the cathodic polarization curve is thought to be due to changes in thermodynamics or kinetics of the cathodic reduction reaction as influenced by changes in biofilm chemistry. It has been widely accepted that ennoblement could result from changes in reversible potential, exchange current density or Tafel slope of the cathodic reaction or passive current density of the metal or a combination of all these[6].

Chandrasekaran and Dexter[1,7] have identified and measured peroxide in biofilms formed over platinum coupons. Peroxide has also been reported[7] to be present in biofilms grown at Tuticorin, India. The thermodynamic contributions of peroxide and pH towards ennoblement have

already been discussed[1]. On the basis of the data collected at the Delaware site, the chemistry at the biofilm-substratum thought to be responsible for ennoblement includes a combination of peroxide with low oxygen and low pH. This chemistry can develop by a combination of biotic and abiotic reactions that take place in a biofilm. Biofilms have the capability to alter the interfacial chemistry through biotic or abiotic mechanisms or both. Each of these in turn, changes the thermodynamics and kinetics of the cathodic reaction, thus, contributing to ennoblement. The significance of these changes lies in the fact that a noble shift in OCP leads to an increased probability of localized corrosion initiation[8]. Experimental investigations were carried out in this work to identify the relative contributions of thermodynamic and kinetic factors that contribute to ennoblement.

Experimental Procedure

Biofilms were developed on platinum and stainless alloy UNS: S44660 (nominal composition: Cr 27.36%, Ni 2.02%, Mo 3.53%, C 0.25%, balance Fe) using once-through flowing seawater at the College of Marine Studies, University of Delaware research facilities on the Delaware Bay at Lewes. The coupons were polished through 600 grit metallurgical paper to produce a uniform surface finish. Seawater characteristics during the exposure were: temperature 20 to 27^{0}C, pH 7.7 to 8.1, salinity 22 to 32 parts per thousand and oxygen (air saturated) at 5 to 8 ppm. The thickness of the biofilm varied with substratum and time of exposure. The thickness of biofilm on platinum was in the range of 30 to 70 mm (40 to 140 mm on S44660) with macroscopically complete coverage on both. These films are usually too thick to count all the microorganisms using epifluorescence or scanning electron microscopy.

Electrochemical experiments were conducted using a computer controlled potentiostat with standard accessories and cell set up. This equipment was used for cathodic polarizations of various coupons with and without biofilms. Platinum was used for counter electrodes and a saturated calomel electrode (SCE) for the reference. A high impedance digital voltmeter was used to monitor the OCP. Cyclic voltammetry (CV) was carried out on biofilmed platinum and stainless alloy coupons using a computer controlled potentiostat. The results were directly imported into a graphics software package for data manipulation. AC impedance was carried out, using a computer

controlled potentiostat with a two phase lock-in analyzer. Both single- and multi-sine techniques were adopted.

A standard graphical technique[9], involving back extrapolation, was adopted for determining exchange current density from the cathodic polarization curves of platinum and S44660 stainless alloy samples. A change in the Tafel slope was also determined using the cathodic polarization curves of bare and biofilmed samples. S44660 stainless alloy anodic polarization curves were used for passive current density determination.

Results

Open circuit potentials (OCP) recorded over time for S44660 stainless alloy and platinum in lower Delaware Bay seawater are plotted in Figure 2.1. Each data point represents an average of at least 10 coupons. Cyclic voltammograms carried out at a scan rate of 50 mV/sec on platinum and S44660 stainless coupons are shown in Figures 2.2 and 2.3 respectively. The peroxide oxidation peak appeared in Figure 2.2 at 0.18 V SCE, whereas the oxygen reduction peak was close to -0.2 V SCE. The same peaks appeared in Figure 2.3 at 1 V SCE and -0.8 V SCE. The potential range of scan for S44660 stainless was different than for platinum. Reasons for the larger range in potential between these peaks on stainless as compared to platinum are discussed in the next section. For the S44660 stainless voltammogram, the peroxide peak was also sharper than the equivalent peak on platinum, and the peak was more prominent for the coupons with lesser degrees of ennoblement (Figure 3.3).

Cathodic polarization curves for both platinum and S44660 stainless alloy samples are shown in Figures 2.4 and 2.5 respectively. The curves were shifted progressively toward more noble potentials and higher current densities as the biofilm matured. The cathodic reaction on any passive alloy exposed to seawater involves oxygen reduction. Exchange current densities for the oxygen reaction were determined from these curves by back extrapolating the Tafel line to the steady state corrosion potential. The results as presented in Table 2.1 indicate that the exchange current density values increased with biofilm formation and maturation. All the biofilmed coupons

in Figure 2.5 had the same duration of exposure, and the differences in ennoblement are presumed to be due to variations in the rate of biofilm development from sample to sample.

Anodic polarization[10] curves for S44660 stainless alloy (Figure 2.6) were measured using a scan rate of 0.6 V/hr to determine the passive current density, i_p. Thus, the curves were terminated at 850 mV before reaching the pitting potential. The passive current densities for both bare and biofilmed samples fell within the same scatter band from 1 to 3.9 X 10^{-2} A/m^2. These data indicate that changes in passive current density with biofilm development do not contribute significantly to ennoblement. Similar data were also observed for platinum (Data not shown).

The electrochemical impedance spectra of biofilmed and bare platinum are shown in the form of a Bode plot in Figure 2.7. It can be seen from this figure that biofilms decreased the phase angle and polarization resistance and increased the double layer capacitance of the samples. For a two month old biofilm the phase angle, polarization resistance and capacitance were almost unchanged from that of the bare electrode. The five month old biofilm had the lowest phase angle and polarization resistance and the highest capacitance, with the two year biofilms being intermediate in all three values.

Discussion

Figure 2.1 showed the ennoblement of S44660 stainless alloy and platinum. The onset of ennoblement on platinum was delayed due to macrofouling right from the start of exposure by amphipods, which built a clay nest on each coupon. However, due to seasonal changes the amphipods disappeared from the water and also from the coupons.

Normally the cathodic reaction on passive metals exposed to aerated seawater is reduction of oxygen to OH^-, particularly at potentials too noble for hydrogen evolution. Under these conditions it is well known that a reduction in pH of the solution causes a noble shift in OCP. Theoretically, that shift is 60 mV per unit drop in pH. The actual shift is less than that due to the irreversible nature of oxygen electrode[11]. In addition, Chandrasekaran and Dexter have recently

measured hydrogen peroxide in marine biofilms[1]. Since the thermodynamic potential for the peroxide reduction reaction is about 500 mV more noble than that for oxygen, peroxide can also contribute to ennoblement[1,12,13]. The recent measurement of peroxide in biofilms at a different experimental site in India[7] supports the idea that peroxide is an important contributor to ennoblement.

Figures 2.2 and 2.3 showed the oxygen and peroxide peaks from cyclic voltammograms of both bare and biofilmed coupons of platinum and S44660. A larger voltage range was needed to reproduce these peaks on the stainless alloy than on platinum (see Figure 2.3). The reason for this is thought to be related to a slower charge transfer across the stainless interface as compared to platinum. At these fast scan rates, the slower charge transfer would shift the peak to a higher value of potential. The peroxide peak on stainless alloy S44660 was also sharper than its equivalent on platinum, and the down slope side of the peak seems to be cut off. We think this is an artifact caused by oxygen evolution starting at about the peroxide peak potential during the scan on S44660. Since both oxygen and peroxide peaks were found on S44660 as well as platinum, this confirms the importance of the oxygen reduction system (of which peroxide is also a component) in the ennoblement process on both metals.

It was seen from the data in Table 2.1 that the Tafel slope, exchange current density and passive current density all changed as the biofilm matured. The changes in passive current density were neither large nor systematic, and it is felt that they probably had little effect on ennoblement. The shifts in exchange current density, however, were significant. The term, exchange current density, as applied to these biofilmed electrodes refers to an overall apparent value representative of the sum of the individual exchange currents for the redox reactions taking place on the surface. In the case of bare platinum, the exchange current density would correspond to the reduction of dissolved oxygen to OH^-. The hydrogen reduction reaction is not thermodynamically possible at these ennobled potentials. In the presence of the biofilm, particularly at low pH, several reduction reactions are possible. We envision the reaction shifting from predominantly oxygen, to a combination of oxygen and peroxide, to predominantly peroxide as the biofilm develops. In a mature biofilm other redox systems may also be possible. In thick biofilms, oxygen ingression by

diffusion is known to be limited to the outermost 20 μm of the film due to oxygen utilization by the organism's metabolism[14]. This limitation on availability of oxygen would favor a predominance of the peroxide reduction alone at the metal surface under biofilms thicker than 20 μm. Even in this case, however, reduction of peroxide would produce some oxygen at the metal surface.

It is important to note that the exchange current density is a strong function of the electrode composition and surface roughness, the presence of trace impurities, the ratio of oxidized and reduced species present and the temperature [15,16]. Yeager[17] observed that the exchange current density for the oxygen electrode is very low, typically 10^{-6} to 10^{-7} A/m^2 on an effective catalytic surface such as platinum at room temperature. Hoare[18] gave a value of 10^{-5} to 10^{-6} A/m^2 for the reduction of oxygen on platinum in acid solutions. Yeager[19] observed high exchange current densities for the oxygen-peroxide couple. For example, an exchange current density on the order of 1 A/m^2 for oxygen at 1 atmospheric and a peroxide concentration of 10^{-4} M in 4M KOH at 60^{0}C was observed[19].

For a bare platinum electrode in well aerated seawater the exchange current density in this work was about 2.5 X 10^{-4} A/m^2. In the presence of a mature biofilm the exchange current density increased to 4.5 X 10^{-3} A/m^2. This shift in the exchange current value could be due to the presence of peroxide in the biofilm. A larger shift in exchange current density was observed for S44660 stainless alloy than for platinum. The exchange current density shifted from 1 X 10^{-5} A/m^2 for a bare electrode to 5 X 10^{-3} A/m^2 for a mature biofilmed electrode. This leads to an important conclusion about the difference between the way biofilms contribute to ennoblement on different substrata. Due to the already high exchange current density for oxygen on platinum, the increase in exchange current density in the presence of biofilms was larger on S44660 alloy than on platinum. Thus, a balance of thermodynamic (change in oxygen potential) and kinetic (increase in exchange current density) factors produces the observed ennoblement on stainless alloy S44660, while the thermodynamic factor is more dominant on platinum. Another kinetic factor, the cathodic Tafel slope also increased on both metals (See Table 2.1). An increase in the cathodic Tafel slope may signify the reduction of peroxide in a biofilm. Higher cathodic Tafel slope has also been observed for peroxide reduction than oxygen reduction on platinum in acid solutions[20].

From this work and all our previous work[1,7,11], we feel thatlow pH and peroxide together with low oxygen concentration bring about a change in thermodynamics and kinetics of the cathodic reaction on biofilmed electrodes. From the electrochemical impedance spectra for the bare and biofilmed platinum samples it can be inferred that the biofilms lower the polarization resistance of the samples. This may be accomplished by rapid electron transfer by the macromolecules known to be present in biofilms[21,22]. The five month old biofilm had a lower polarization resistance and higher double layer capacitance than the other biofilms. This might be due to differences in the rate of development, distribution, and composition of the biofilm.

Summary

Biofilms bring about ennoblement by changing the thermodynamics and kinetics of the cathodic reaction. Depending upon the nature of the substratum the relative contributions of thermodynamics and kinetics may vary. For S44660 stainless alloy ennoblement is brought about by balanced contributions from both kinetics and thermodynamics, whereas thermodynamic effects dominate on platinum. The predominant mechanism of cathodic depolarization due to biofilm formation involves various species of the oxygen reduction system (e.g. peroxide). Biofilms appear to lower the polarization resistance and enhance rapid electron transfer between the substratum and the electrolyte.

Acknowledgments

This work was sponsored by The Office of Naval Research under contract number 01490J1947. We also acknowledge Dr. D.C. Hansen for his help in interpreting the AC impedance data.

References

1. P. Chandrasekaran and S.C. Dexter, CORROSION/93, Paper 493, (Houston, TX: NACE, 1993).
2. O. Varjonen, T. Hakkarainen, E.N. Lassila and M.S. Salonen, In the Proceedings of the 11th Scandinavian Corrosion Congress, Stavanger, Norway, paper no. F-67, June 19-21, 1989.
3. R.A. Buchanan, P. Li, X. Zhang, N.D.E. Dowling, E.E. Stansbury, J.C. Danko and D.C. White, "Electrochemical Field Studies of Freshwater Microbiologically Influenced Corrosion", A Progress Report to the EPRI, Dec. 1989.
4. S. Matoda, Y. Suzuki, T. Shinohara and S.Tsujikawa, Corrosion Science, 31(1990): pp. 515-520.
5. H-J Zhang and S.C. Dexter, In the Proceedings of the 12th International Corrosion Congress, (Houston,TX: NACE, 1993) Vol. 5B, pp. 3761-3772.
6. S.C. Dexter, Biofouling, Vol. 7(1993) pp. 97-127.
7. P. Chandrasekaran and S.C. Dexter, In the Proceedings of the 12th International Corrosion Congress (Houston, TX: NACE, 1993) Vol. 5B, pp. 3696-3707.
8. M. Urquidi and D.D. Macdonald, J. Electrochem. Soc, Vol. 132 (1985), p. 555.
9. D.A. Jones, in Principles and Prevention of Corrosion, (Macmillan Publishing Company, New York, 1992) pp. 76.
10. Annual book of ASTM standards, Section 3, Metals Test Methods and Analytical Procedures, (Vol. 03.02, Wear and Erosion; Metal Corrosion, 1989), Designation G5-87, pp. 79-85.
11. S.C. Dexter, H.-J. Zhang and P. Chandrasekaran, Biofouling effects on corrosion of stainless alloys in seawater, In Biotoxins, Biodegradation and Biodeterioration Research IV, Eds. C. O'Rear, G. Llewellyn, W. Dashek, (Plenum press, 1993), pp. 596-617.
12. M. Pourbaix, in The Atlas of Electrochemical Equilibria in Aqueous Solutions, (Houston, TX: NACE,1974) pp. 97-111.
13. G. Salvago, G. Fumagalli, G. Taccani, P. Cristiani and G. Rocchini, "Electrochemical and corrosion behavior of passive and fouled metallic materials in seawater", In European Federation of Corrosion Publications, No.8, Microbial Corrosion, Proceedings of the 2nd EFC Workshop Portugal, 1991. Eds. C.A.C. Sequeira and A.K. Tiller, Published for the European Federation of Corrosion by the Institute of Materials, 1992.
14. J.W.T. Wimpenny, A. Peters and M. Scourfield, Modeling spatial gradients, In Structure and Function of Biofilms Eds. W.G. Characklis and P.A. Wilderer, (John-Wiley and Sons Ltd, 1989), pp. 111-127.
15. B.D. Craig in Fundamental aspects of Corrosion Films in Corrosion Science, (Plenum Press, New York, 1991), p. 28.
16. K.B. Oldham and C.G. Zoski, Chapter 2 in The Electrode Kinetics: Principles and Methodology, Vol. 26 of Comprehensive Chemical Kinetics, Eds. C.H. Bamford and R.G. Compton, (Elsevier Publishers, 1986), p. 105.
17. E. Yeager, U.S. NTIS, AD Rep., AD-A042487, (Gov. Rep. Announce. Index (U.S) 77(21), 113, 1977), p. 28.
18. J.P. Hoare, in The Electrochemistry of Oxygen, (Interscience Publishers, New York, 1968).
19. E. Yeager, Journal of Molecular Catalysis, 38, (1986): pp. 5-25.
20. J.P. Hoare, in Electrochemistry of Oxygen, (Interscience Pub., New York, 1968), p. 162.
21. B.E. Christensen and W.G. Characklis, Chapter 4 in Biofilms, Eds. W.G. Characklis and K.C. Marshall (A Wiley-Interscience Publication, New York, 1990), pp. 93.
22. S. Isied, Chapt 15 in Electron Transfer in Inorganic, Organic & Biol. Sys., J.Bolton, N. Mataga, G.McLendon, Eds. Adv. in Chem. Series (Amer. Chem. Soc., Wash., D.C., 1991), p. 229.

Table 2.1 Electrochemical parameters measured from cathodic and anodic polarization curves for alloy 44660 and platinum.

Parameter	Bare coupon	Biofilmed Coupon
Exchange current density for S44660 (A/m^2)	**1×10^{-5}**	**5×10^{-3}**
Exchange current density for platinum (A/m^2)	**2.5×10^{-4}**	**4.5×10^{-3}**
Cathodic Tafel slope for S44660 (V/decade of current density)	**0.12**	**0.40**
Cathodic Tafel slope for platinum (V/decade of current density)	**0.09**	**0.24**
Passive current density for S44660 (A/m^2)	**1×10^{-2}**	**1×10^{-2} to 3.9×10^{-2}**
Passive current density for platinum (A/m^2)	**1.6×10^{-2}**	**6.3×10^{-3} to 3.2×10^{-2}**

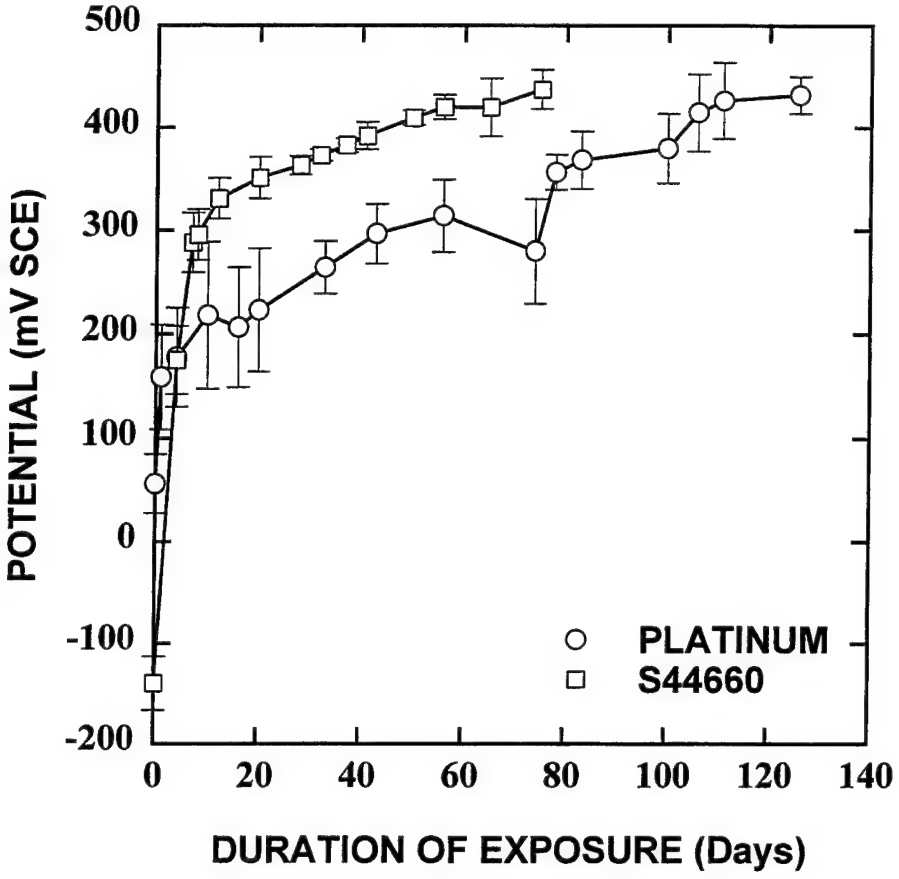

Figure 2.1 Open circuit potential ennoblement data for platinum and stainless alloy S44660 in Lower Delaware Bay seawater.

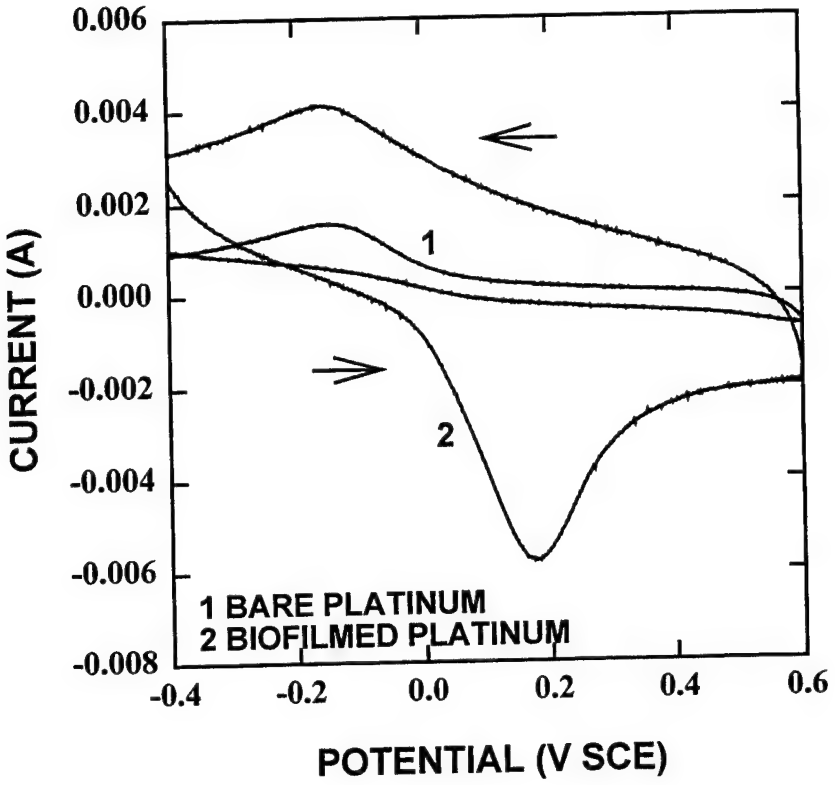

Figure 2.2 Cyclic voltammograms of platinum coupons in natural seawater. The scan rate was 50 mV/sec, and the arrows indicate the scan direction. The biofilmed coupon was ennobled to 450 mV SCE.

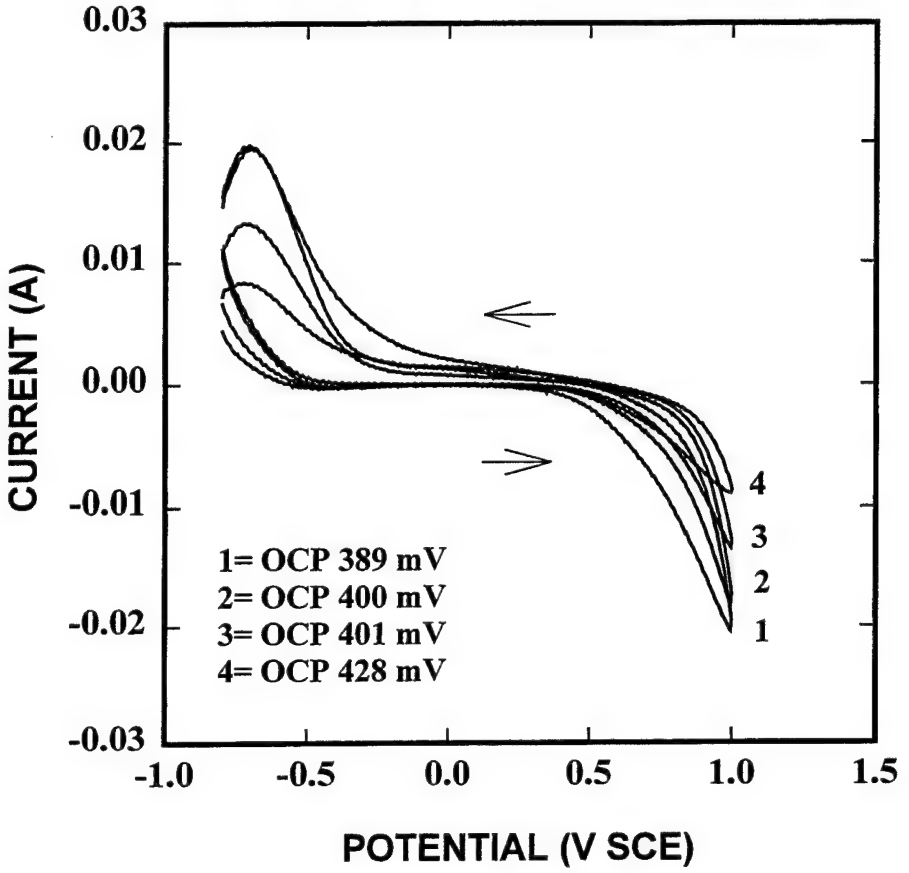

Figure 2.3 Cyclic voltammograms of biofilmed stainless alloy S44660 ennobled to different OCP values in natural seawater after exposure for 2 months in Delaware Bay seawater. The scan rate was 50 mV/sec, and the arrows indicate the scan direction.

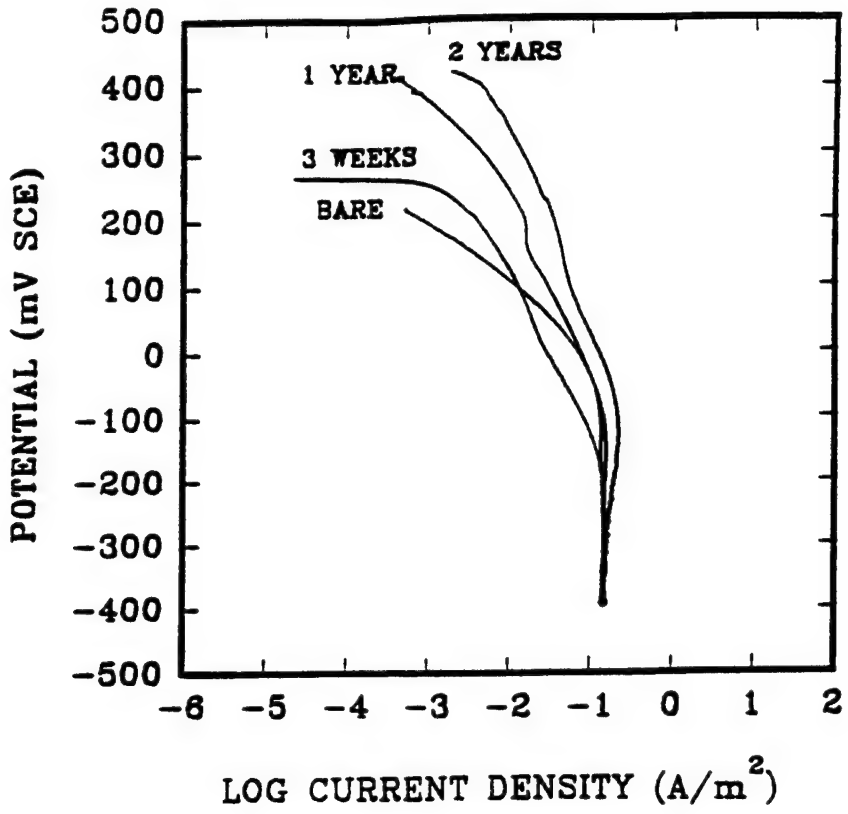

Figure 2.4 Cathodic polarization curves for platinum in natural seawater. Durations of exposure for each coupon are as marked.

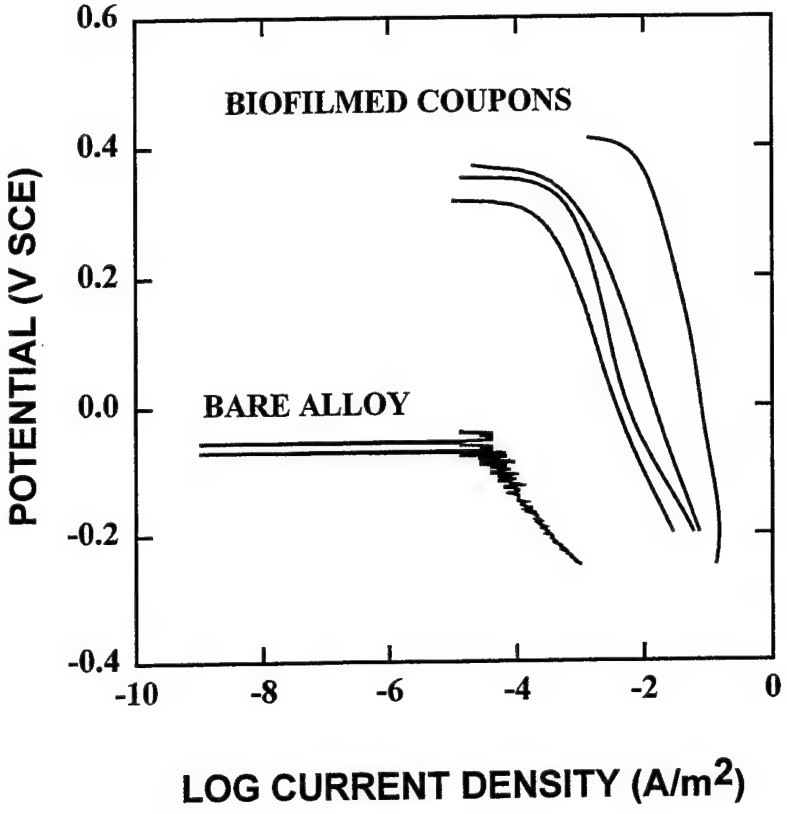

Figure 2.5 Cathodic polarization curves for stainless alloy S44660 in natural seawater. The biofilmed coupons were all exposed for two months in Delaware Bay seawater, but they were ennobled to different levels of potential.

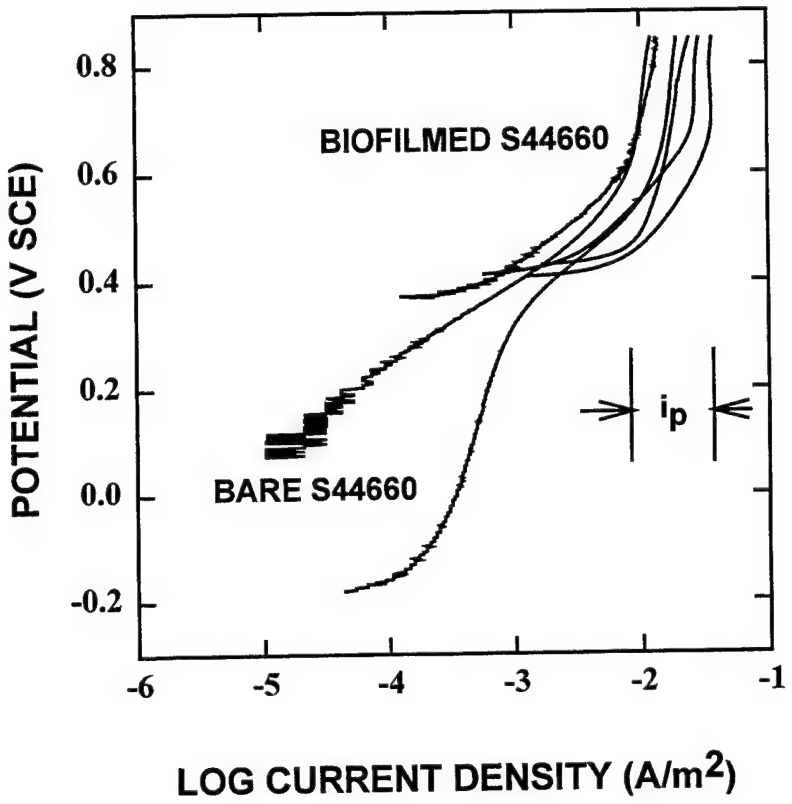

Figure 2.6 Anodic polarization curves for two bare and four biofilmed stainless alloy S44660 coupons. All biofilmed coupons were exposed for two months in Delaware Bay seawater. Note that all six values of i_p fall randomly within the same scatter band.

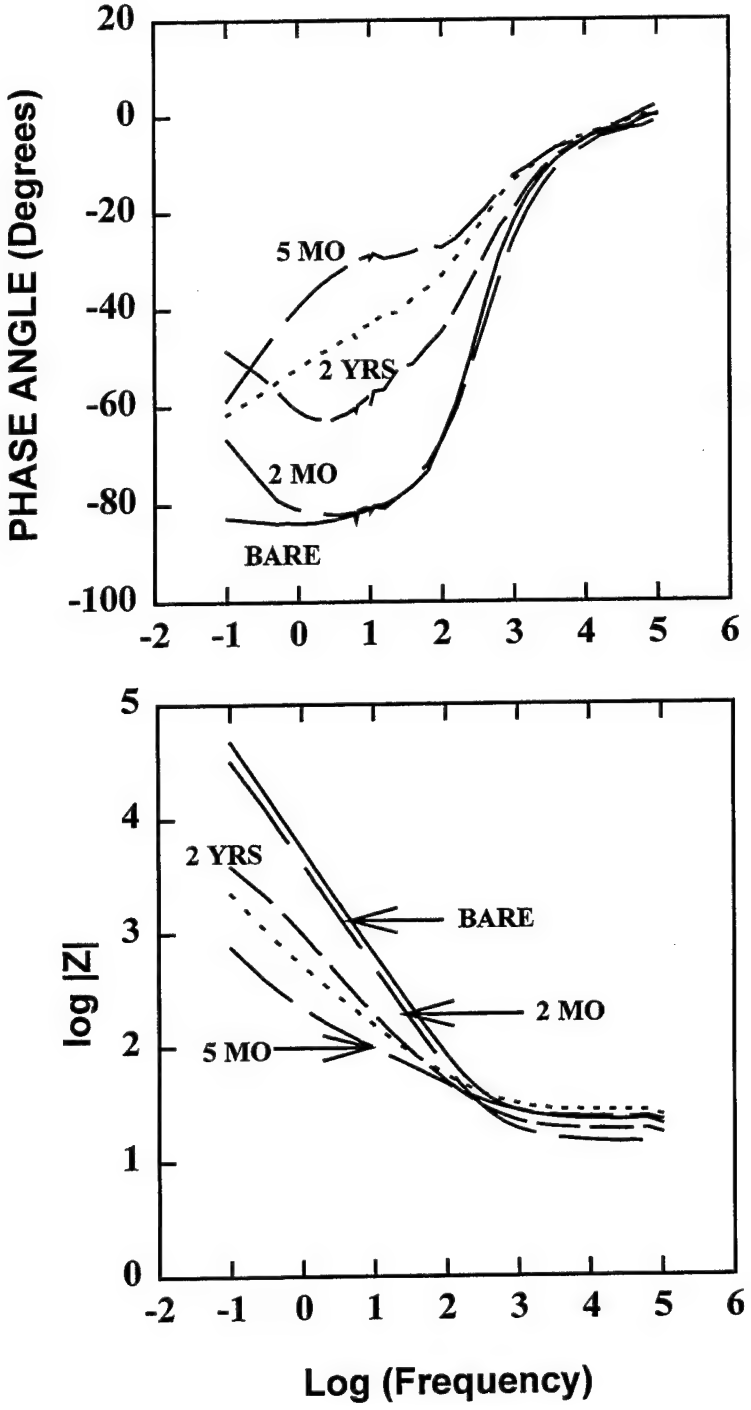

Figure 2.7 Electrochemical impedance spectra (Bode plot) for bare and biofilmed platinum samples exposed as indicated in natural seawater.

Chapter 3

Biofilm Consortia and the Role of Iron and Manganese in OCP Ennoblement

Abstract

The natural assemblage biofilms developed on platinum are complex in their structure and diverse in bacterial community. Utilization of various electron acceptors such as oxygen, iron, manganese etc., are thought to be important for ennoblement to take place on platinum. This paper addresses as to the existence of anaerobic group of bacteria such as iron and manganese utilizers and the evidence is brought out in terms of these elements mineralized in natural biofilms. Surface analytical and micro-electrode techniques have been used to identify and characterize the elements present. Inductively coupled atomic emission spectroscopy was used to measure the concentration of these elements. The importance of the presence of heavy metals and their role in promoting ennoblement and thereby causing accelerated corrosion are also discussed.

Introduction

Development of biofilms on any solid substratum exposed to seawater is a matter of time. The environmental conditions dictate the bacterial diversity and the population dynamics. However, once the biofilms develop and mature there are regions within the biofilm where microniches develop, and widely different groups of bacteria can coexist.

Biofilms developed over passive metals are known to ennoble the open circuit potential (Mollica and Trevis, 1976; Mollica et al. 1987; Johnson and Bardal, 1985; Johnson and Bardal, 1986; Dexter 1993; Chandrasekaran 1995). Establishment of ennoblement and the absolute value of the ennoblement in seawater are dependent upon various factors such as the bacterial diversity (Maruthamuthu et al; 1993), biofilm thickness (Zhang, 1993), environmental factors like sun light and temperature (Dexter and Zhang., 1991; Maruthamuthu et al., 1992, Maruthamuthu et al., 1995; Eashwar et al., 1995), the nutrient condition of the supporting water etc., The factors mentioned above will also dictate the chemistry of the biofilm and its capacity to bring about the ennoblement. It has already been shown by various authors that the metabolism of bacteria, and not their mere presence alone is the reason for ennoblement (Scotto, 1985; Mollica and Trevis., 1976).

Biofilms developed at the Lower Delaware Bay test facilities are known to contain gram positive, gram negative and eucaryotic groups of microorganisms based on the PLFA analysis conducted at the University of Tennessee by Dr. Peter Angell and his coworkers (Chandrasekaran, 1995). Experiments conducted at our laboratory have also shown the presence and the importance of aerobic and anaerobic groups of microorganisms (Chandrasekaran and Dexter, 1994). Extensive work was also carried out to identify the importance of each group of bacteria to the ennoblement mechanism (Chandrasekaran, 1995).

The presence of peroxide (Chandrasekaran and Dexter, 1993a), heavy metals (Chandrasekaran and Dexter 1994; Chandrasekaran, 1995), and low oxygen in biofilms has been verified (Lewandowski et al., 1988; Chandrasekaran, 1995). This paper addresses a part of the biofilm chemistry created due to bacterial metabolism and the methods devised to qualitatively and quantitatively measure it. Biofilms developed on platinum at our site have shown a combination of red, brown and yellow colors. The change in color of these biofilms was thought to be due to accumulation of the heavy metals, iron and manganese, and mineralization of these heavy metals either as an oxide or as a sulfide (Chandrasekaran and Dexter 1994). However, the dimension of the biofilm and the delicate nature of the interfacial chemistry makes it challenging to study the contributory factors for the chemistry.

Experimental Procedure

The Lower Delaware Bay seawater used for our experiments supports a wide variety of microorganisms and chemical processes. On the in-coming tide it is essentially coastal Atlantic seawater of salinity 28 to 30 parts per thousand. On the out-going tide it is the effluent from the local tidal wetlands or salt marshes. Salt marshes are known for their ability to support a variety of chemical and biological reactions involved in heavy metal and sulfur cycling, and it is probable that the microorganisms involved in these reactions are abundant in both the water and biofilms used in our experiments. The water itself is also high in both dissolved organics and suspended particulates.

Platinum was used in developing natural biofilm consortia. The surfaces of the coupons were polished through 600 grit silicon carbide metallurgical paper for a reproducible surface finish. Biofilmed coupons were used for further investigations. A high impedance digital volt meter was used to record the open circuit potential of the platinum coupons exposed to natural seawater.

The color of biofilms formed on platinum at our location changed from clear to brown, red and yellow in appearance over several months. The color of the biofilm on platinum provided clues for identifying the elements present in the film and also the bacterial metabolism that is responsible for it. A Philips model 501 Scanning electron microscope (SEM) with Energy dispersive X ray analyzer (EDAX) model 9100 was used for elemental analysis of biofilmed coupons ranging in age from one week to two years. Biofilmed platinum coupons from natural seawater exposure were dehydrated in a vacuum chamber for about 30 minutes without any other pretreatment or coating. The imaging capability of SEM was used to choose the location on the surface, and EDAX was used to identify the elements present in the biofilm at that location. Types of locations chosen varied from a general section of the biofilmed surface with as few particles as possible to the particles themselves. The particles fell into two general categories. First were suspended particulates from the water that had become trapped in the biofilm matrix. These were easy to distinguish because of their relatively large size and their tendency to charge up under the electron beam. For the most part, these particles were avoided. The second type of particles were smaller and more crystalline in appearance.

Environmental scanning electron microscopy with additional energy dispersive X-ray analyses were carried out by Dr. Brenda Little, Dr. Patricia Wagner and Dr. Richard Ray of Naval Research Laboratory, Stennis Space Center, MS on a platinum biofilmed coupon sent from our site (Chandrasekaran, 1995). The platinum coupon was exposed for 15 months in natural seawater for biofilm formation. The coupon also had the characteristic colors, indicating presence of heavy metals. The purpose of this analysis was to determine the relation between the groups of bacteria and the heavy metals known to be present in these biofilms. There was a small problem in shipping the biofilm to the laboratory and hence the relationship between the bacteria present in the biofilm and the heavy metals could not be carried out. However, the EDAX results can be used as an independent measurement of heavy metals presence in these biofilms.

In order to get data on the heavy metal concentrations in biofilms developed over platinum, we have used Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). The ICP analyses were carried out as described below using a Jobin-Yvon 70 Plus instrument operating at 1.2 KW. The emission peaks of iron and manganese were calibrated at 238.204 and 257.610 nm wavelengths. This technique measures total metals regardless of oxidation state or binding.

The following trace metal handling procedures (Bruland and Franks, 1979) were used to acid clean polyethylene centrifuge tubes in order to make that they introduced no heavy metal contamination. Tubes were washed in tap water with phosphate free detergent and rinsed in deionized water and acetone. They were then rinsed in deionized water before soaking for three days in 5N HNO_3, rinsed once again in deionized water and soaked in 3 N HCl for another three days. Next they were rinsed in deionized water and transferred to 0.1N ultrapure HCl for three more days. Finally, they were rinsed in deionized water and dried in a laminar-flow clean air bench. Biofilmed and ennobled platinum coupons exposed for various time periods in natural seawater were placed directly in these centrifuge tubes, and 5 ml of ultrapure concentrated nitric acid were added to digest the film along with any heavy metals therein. For comparison, the blank consisted of a bare platinum electrode rinsed with deionized water and immersed in 5 ml of the same ultrapure nitric acid in one of the precleaned polyethylene centrifuge tubes.

The ICP instrument was calibrated for iron and manganese using several standards. This procedure was necessary due to the low concentration levels expected to be measured in the biofilms. This procedure also ensured the reliability of the measured concentrations of iron and manganese. The instrument normally uses less than 1 ml of the sample and takes three measurements. It then gives the mean and standard deviation for each run. The heavy metal concentrations measured directly by the ICP were then converted to molar concentrations in the biofilm by correcting for the difference in volume between the biofilm and the solution in the centrifuge tube. The thickness of the biofilm was measured using a stereo microscope sequentially focusing on the top and bottom of the film and recording the travel of the microscope head. The final thickness for each film was taken as the average of six readings at randomly selected areas.

It was assumed that all the heavy metals from the biofilm were released into the 5 ml ultrapure nitric acid, and that the concentration measured in this volume of acid must have come from the biofilm alone. A mathematical procedure, using the measured values of concentration of heavy metals, volume of biofilm and the 5 ml of ultrapure nitric acid, was adopted for converting the ICP concentrations to concentrations in the biofilm. The measurements were used first to identify the time at which heavy metals began to deposit, and second to observe changes in concentration with increasing exposure time. The time of initial deposition would show approximately when the biofilm starts using iron and manganese as electron acceptors. The increase in concentration of these metals over time would show rates of accumulation.

RESULTS

Figure 3.1 shows the effect of super-saturating the bulk seawater with oxygen surrounding a biofilmed and ennobled electrode. Control experiments were carried out using a biofilmed electrode exposed to natural seawater with the dissolved oxygen at air saturation. Exposure of the ennobled electrode to a higher oxygen concentration than atmospheric in the bulk seawater shifted the OCP values to more active potentials, whereas the OCP of controls remained relatively constant. Figure 3.2 shows the effect of deaerating the bulk seawater surrounding the biofilmed and ennobled electrode. Here too the control remained unaffected, whereas the OCP of the experimental samples dropped to negative values over a period of time. This signifies the importance of bulk oxygen concentration as an important factor for ennoblement.

Typical EDAX data are shown in Figures 3.3 to 3.7 for elements present in young (one week) and mature (up to 24 months) biofilms developed on platinum coupons immersed in natural seawater. These EDAX spectra were taken from an area of the general biofilm matrix having as few large deposits and particles as possible. Thus, they represent the elemental composition of the biofilm matrix. It is apparent from these spectra that iron and manganese are important at an early stage of biofilm development. All other elements shown were present either in the seawater or in the substratum. Accumulation of iron and manganese in the biofilm also coincided with changes in color of the filmed coupon from silvery to a combination of red, brown and yellow.

In an effort to identify the stoichiometry of individual deposits, SEM was used to select a biofilm location rich in particles and to focus on an individual solid particle for EDAX analysis. Figure 3.8 shows the EDAX from one such solid particle of dimension close to 9 μm irradiated by an electron beam of 0.25 μm diameter. EDAX showed a higher concentration of iron than is usually observed in the general biofilm matrix (Figures 3.3 to 3.7) along with high concentrations of silica and calcium. A larger particle (20 by 47 μm) had high concentrations of iron and silicon (Figure 3.9). Even though a clear picture of stoichiometry of the deposits is not available, the data show that heavy metal accumulation takes place in a biofilm. Figures 3.10 and 3.11 show the EDAX data collected together with the ESEM work. This data serve as an independent confirmation to the presence of iron and manganese in marine biofilms developed at our site.

Inductively coupled plasma atomic emission spectroscopy was used to quantify the iron and manganese that were accumulated in marine biofilms on platinum. In spite of the careful handling procedures, the blank showed small heavy metal concentrations (1.16 mM iron and 0.02 mM manganese), which were subtracted from the experimental values. The concentrations of iron and manganese on a bare coupon and in biofilms formed for 2 to 24 months did not follow any trend with respect to thickness, age of the biofilm, or the OCP of platinum coupon (Table 1). However, it is important to note that iron and manganese accumulation began within the first two months of exposure to natural seawater. The reasons for the accumulation of iron and manganese and their effect on biofilm chemistry are important for understanding the ennoblement mechanism.

DISCUSSION

A mature biofilm is expected to have aerobes, microaerobes and anaerobes working together in creating a favorable chemistry at the biofilm-substratum interface for ennoblement. In such a biofilm it is reasonable to expect that a higher concentration of oxygen in the bulk water would create a correspondingly higher concentration of oxygen within the biofilm. This would inactivate the obligate anaerobic group of microorganisms resident in the film, or at least shrink the thickness of the anaerobic zone. Higher oxygen concentration should also increase peroxide in the aerobic portions of the biofilm, possibly deactivating the aerobic group due to the toxicity of the

peroxide. In both cases above the OCP should shift to less noble values, and this is exactly what is observed in Figure 3.1. In a similar way, sparging the bulk water with helium to a dissolved oxygen concentration less that 0.1 ppm also caused the OCP values to become less noble, as was shown in Figure 3.2. Under these conditions only the anaerobic bacteria in the biofilm would have been active. These results reinforce the idea that a consortia of different types of microorganisms is needed for ennoblement, rather than any single group of microorganisms.

Iron and manganese were measured in the mM range in marine biofilms (Table 1), whereas they are present in nM concentrations in open ocean waters (Wu and Luther, 1994). Bacterial metabolism is the only means that can explain the six orders of magnitude difference in concentrations of iron and manganese between biofilm and seawater. Based on all the above data, it can be said that in a natural assemblage biofilm over a period of time many electron acceptors are present and all of them may contribute to for ennoblement.

The presence of heavy metals in a natural assemblage biofilm on a platinum coupon might be the reason for the characteristic colors that are developed. If the oxidation states of iron and manganese present in the biofilm are known then pH in a biofilm can be estimated indirectly using potential-pH diagrams (Pourbaix, 1974). Iron and manganese are known to exist in a number of oxidation states. Since there is an oxidizing and reducing environment present simultaneously in a biofilm, the oxidized heavy metals would be precipitated at the oxic layer due to presence of peroxide and oxygen, whereas the reducing conditions prevailing at the metal biofilm interface would reduce these heavy metals and make them more soluble.

The iron and manganese deposits in a biofilm on platinum create a condition akin to corrosion products adhering to stainless steel or carbon steel surface in seawater. This is similar to an occluded cell condition. The access of electrolyte is restricted, due to the spatial geometry of the deposits adhering to the surface. There are various examples in the corrosion literature for the hydrolysis of heavy metals and creation of acids (Pourbaix, 1974; Streicher, 1974; Fontana, 1986). Occluded cell corrosion at pits and crevices is one form of corrosion in which heavy metal hydrolysis is often encountered. In the above examples it is the heavy metals in the corrosion product that become hydrolyzed, whereas in the biofilm it is the heavy metals accumulated due to

bacterial metabolism that become hydrolyzed. This is the major difference in these two cases. Rosenfeld (1974) showed a range of pH values starting from 0.71 to 6.64 is possible in a stainless steel crevice corrosion solution as a result of hydrolysis of various corrosion products. Nash and Kelly (1993) reported that the crevice corrosion solution developed over type 304 stainless steel is high in metal and chloride ions with pH less than 2.

Streicher (1974) observed that bacteria deposited manganese from water in heat exchangers. The deposited manganese subsequently oxidized to permanganate and hydrolyzed to produce an acidic condition. Streicher (1974) used this information to devise an accelerated test to evaluate the performance of stainless steels. This shows that bacterially produced manganese can also act as an acid producer in a biofilm. The purpose of all the above arguments is to indicate the possibilities that should be considered for decreasing the pH in a biofilm. Bacterially reduced iron and manganese can diffuse from the anoxic portions of the biofilm, become oxidized at the oxic interface and produce an acid concentration. This acid can diffuse both into and out of the film. Whatever diffuses into the film gets trapped, changing the local chemistry of the biofilm-substratum interface. The acid that is present at the metal-biofilm surface can contribute to ennoblement. In a complex marine biofilm both direct production of acidity by the microorganisms and heavy metal hydrolysis can contribute to the total acidity at the biofilm-substratum interface.

Now, based on the chemistry outlined above a decrease in one unit of pH would contribute a maximum of 60 mV towards the thermodynamic reversible potential of the oxygen electrode in seawater. If the average pH of seawater is assumed to be around 8 then a drop to a value of 3, as shown by cyclic voltammetry experiments (Chandrasekaran and Dexter, 1993a; Chandrasekaran and Dexter, 1993b; Chandrasekaran, 1995) would contribute a maximum of 300 mV. The real contribution would be less, because of nonequilibrium conditions.

The presence of peroxide in biofilms is important since peroxide reduction can contribute thermodynamically to ennoblement. Just as peroxide is stabilized for commercial purposes by adding organics together with salicyclic and boric acids (Vogel, 1989), the peroxide produced in a biofilm would be stabilized by the organic materials and acidity present in the film. Under acidic conditions:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \qquad \emptyset = 1.229 \text{ V SHE} \qquad (1)$$

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \qquad \emptyset = 1.776 \text{ V SHE} \qquad (2)$$

Extensive calculations were made for the potential contribution by peroxide under cyclic voltammetry simulation conditions. The Nernst equation gives a thermodynamic potential for reaction (1) as 0.794 V SCE for a pH of 2.9 and a dissolved oxygen concentration of 0.5 ppm. Under the same conditions with a peroxide concentration of 2.4 mM, the potential of reaction (2) is 1.289 V SCE. Thus the presence of peroxide could contribute a thermodynamic maximum of 0.495 V to the ennoblement of the OCP under simulated biofilm conditions. The actual contribution would, of course, be less than the thermodynamic value, but this illustrates that the magnitude of the peroxide contribution under open circuit conditions could be important.

Development of an aggressive interfacial chemistry involving low pH, low oxygen and peroxide should enhance the probability of localized corrosion initiation on low resistance alloys. For example, this type of chemistry may accelerate the initiation and propagation of crevice corrosion as seen by Zhang (1993). There are also some preliminary indications that such an interfacial chemistry may also increase the corrosion of galvanic couples in certain cases (Dexter et al., 1992). Even though seawater acted as the source for accumulating heavy metals on platinum used in the experiments outlined in this paper, stainless steel alloys themselves may act as a source for the heavy metals iron and manganese in some cases, thus making the situation worse.

Conclusions

1. Consortia of both aerobic and anaerobic organisms were required for ennoblement of the OCP in our tests.

2. The bulk water environment can neither be devoid of, nor super-saturated with oxygen for ennoblement to occur.

3. Iron and manganese are present in mM concentrations in the biofilms developed at our site.

4. The actions of oxygen, peroxide and the heavy metals, iron and manganese, appear to be synergistic in that heavy metal cycling and hydrolysis contribute to establishing the low pH environment so conducive to ennoblement.

Acknowledgments

The authors would like to thank the Office of Naval Research for sponsoring this work under the contract number 01490J1947. The authors would also like to thank Prof. G.W. Luther's laboratory for the microelectrode measurements. Dr. Brenda Little and her co-workers are acknowledged for the ESEM and EDAX data. Dr. Peter Angell and his co-workers at Univ. of Tennessee are acknowledged for their PLFA analysis on our biofilms.

References

K.W. Bruland and R.P. Franks, Analytica Chimica Acta, 105, (1979), pp. 233-245.

P. Chandrasekaran and S.C. Dexter, Mechanism of potential ennoblement on passive metals by seawater biofilms, CORROSION/93, Annual Corrosion Conference, Paper No. 493 (Houston, TX: NACE, 1993a).

P. Chandrasekaran and S.C. Dexter, Factors Contributing to ennoblement of passive metals due to biofilms in seawater, In: Proceedings of the 12th Intl. Corrosion Congress, Vol.5B, (Houston, TX: NACE, 1993b), pp. 3696-3707.

P. Chandrasekaran and S.C. Dexter, Bacterial metabolism in biofilm consortia: consequences for potential ennoblement, CORROSION/94, Annual Corrosion Conference, Paper No. 276 (Houston, TX: NACE, 1994).

P. Chandrasekaran, Mechanism of potential ennoblement on passive metals due to biofilms in seawater, Ph.D dissertation, College of Marine Studies, University of Delaware, (Spring 1995), pp. 1-260.

P. Chandrasekaran and S.C. Dexter, Measurements of acid pH within marine biofilms on passive metals (Submitted for publication in Biofouling, 1997).

S.C. Dexter, Role of microfouling organisms in marine corrosion, Biofouling 7, (1993), pp. 97-127.

S.C. Dexter, P. Chandrasekaran, H.J. Zhang and S. Wood., Microbial corrosion in marine environments: Effect of microfouling organisms on corrosion of passive metals, Proc. 1992 Biocorrosion and Biofouling workshop, H. Videla, Z. Lewandowski and R. Lutey (eds.)., Buckman Laboratories, Memphis, TN 1993, p. 171.

S.C. Dexter and H.-J. Zhang, Effect of biofilms, sunlight, and salinity on corrosion potential and corrosion initiation of stainless alloys, EPRI Final Report NP-7275, Research Project 2939-4, 1991.

M. Eashwar, S. Maruthamuthu, S. Palanichamy and K. Balakrishnan, Sunlight irradiation of seawater eliminates ennoblement-causation by biofilms, Biofouling, Vol. 8, No. 3, (1995), pp. 215-221.

M.G. Fontana, Corrosion Engineering, Third Edition, McGraw-Hill Book Company, NY, (1986), pp. 1-544.

R. Johnsen and E. Bardal, Corrosion, 41,5, pp. 296, 1985.

R. Johnsen and E. Bardal, The effect of microbiological slime layer on stainless steel in natural seawater, CORROSION/86, Corrosion Conference, Paper No. 227 (NACE, Houston, TX, 1986).

Z. Lewandowski, W.C. Lee,W.G. Characklis and B. Little, Dissolved Oxygen and pH microelectrode measurements at water immersed metal surfaces, CORROSION/88, Paper No. 93, NACE, Houston, TX.

S. Maruthamuthu, M. Eashwar, S. S. Raja and K. Balakrishnan, Effects of microfouling and light/dark regimes on corrosion potentials of two stainless alloys in seawater, Biofouling, 7, (1993), pp. 257-265.

S. Maruthamuthu, G. Rajagopal, S. Sathianarayanan, M. Eashwar and K. Balakrishnan, A photoelectrochemical approach to the ennoblement process - proposal of an adsorbed inhibitor theory, Biofouling, Vol. 8, No. 3, (1995), pp. 223-232.

A. Mollica and A. Trevis, The influence of the microbiological film on stainless steels in natural seawater, In: Proceedings of the 4th International Congress on Marine Corrosion and Fouling, Juan-Les-Pins, Antibes, France, (1976), p. 351.

A. Mollica, G. Ventura, E. Traverso and V. Scotto, Cathodic behavior of nickel and titanium in Natural Seawater, Proc. 7th Intl. Biodeterioration Symp., Cambridge, UK, 1987.

B.K. Nash and R.G. Kelley, Characterization of the crevice solution chemistry of 304 stainless steel, Corrosion Science, Vol. 35, Nos1-4, (1993), pp. 817-825.

M.Pourbaix, The Atlas of Electrochemical Equilibria in Aqueous Solutions, (Houston, TX: NACE, 1974), pp. 97-111.

I.L Rosenfeld, Crevice corrosion of metals and alloys, In Localized Corrosion, R.W. Staehle, B.F. Brown, J. Kruger and A. Agarwal (eds.) National Association of Corrosion Engineers, Houston, TX, (1974), pp. 373-398.

M.A. Streicher, Corrosion, Vol. 30, No. 3, (1974), pp. 77-91.

Vogel's Text Book of quantitative Chemical Analysis, G.H. Jeffery, J. Bassett, J. Mendham, R.C. Denney (eds.), Fifth Edition, English Language Book Society, Longman Scientific and Technical, Essex, England (1989), pp. 395.

J. Wu and G.W. Luther III, Size-fractionated iron concentrations in the water column of the western North Atlantic Ocean, Limnology and Oceanography, 39, 5, (1994), pp. 1119-1129.

H.-J. Zhang, The effect of biofilms on localized corrosion of stainless alloys, Ph.D dissertation, College of Marine Studies, University of Delaware, 1993.

Table 3.1 Concentrations of iron and manganese in a biofilm formed on platinum over different periods of exposure.

AGE OF BIOFILM (MONTHS)	THICKNESS OF FILM (mm)	CONC. OF Fe In BIOFILM (mM)	CONC. OF Mn In BIOFILM (mM)	OCP VALUE (mV Vs SCE)
0 (Bare Coupon)	None	--	--	158.8± 50.43
2	29	28.72	13.20	251
5	58	25.13	23.28	425
5	44	8.86	21.82	441
24	48	7.33	13.70	463
24	67	10.50	19.21	432

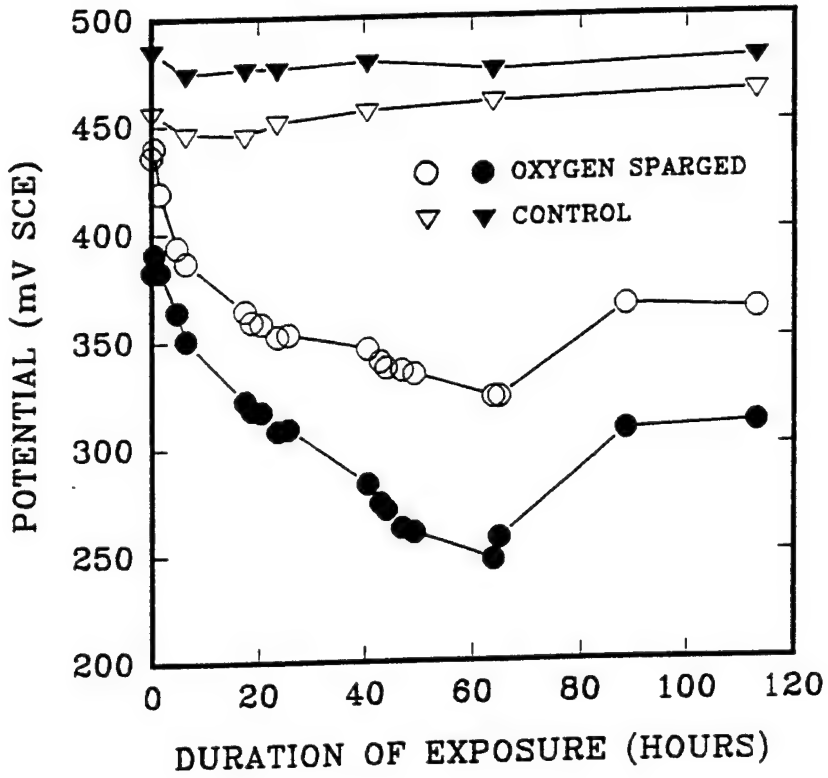

Figure 3.1 Effect of oxygen sparging on OCP of biofilmed and ennobled platinum electrodes vs. control electrodes kept in natural seawater.

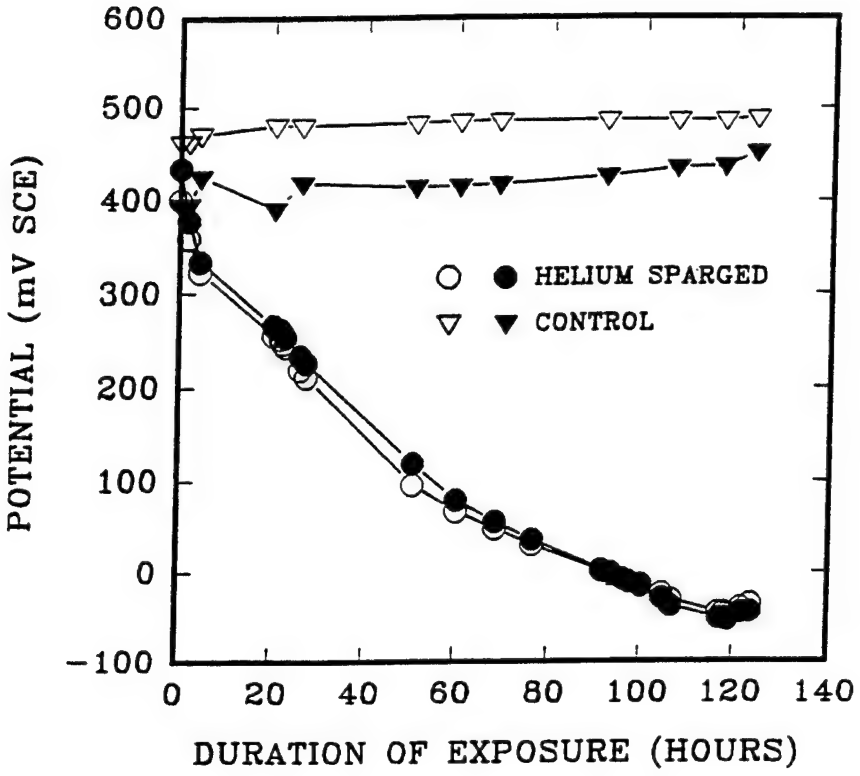

Figure 3.2 Effect of helium sparging on OCP of biofilmed and ennobled platinum electrodes vs. control electrodes kept in natural seawater.

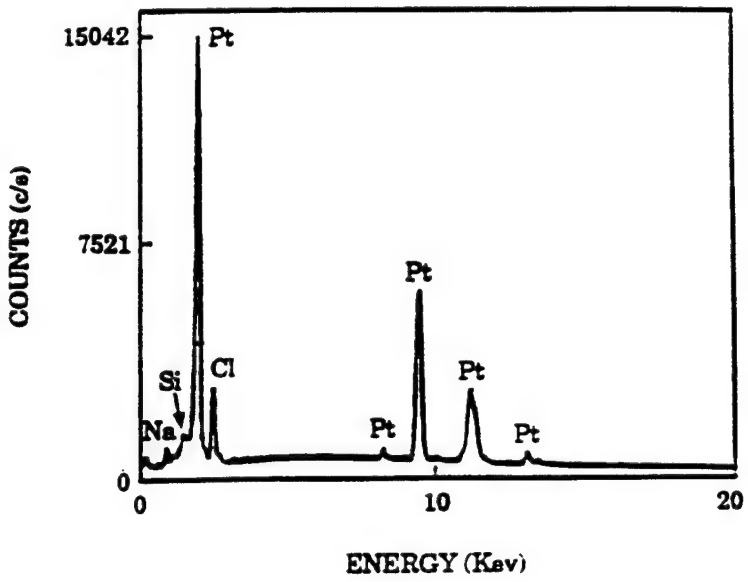

Figure 3.3 EDAX data showing elements present in a one week old biofilm on platinum.

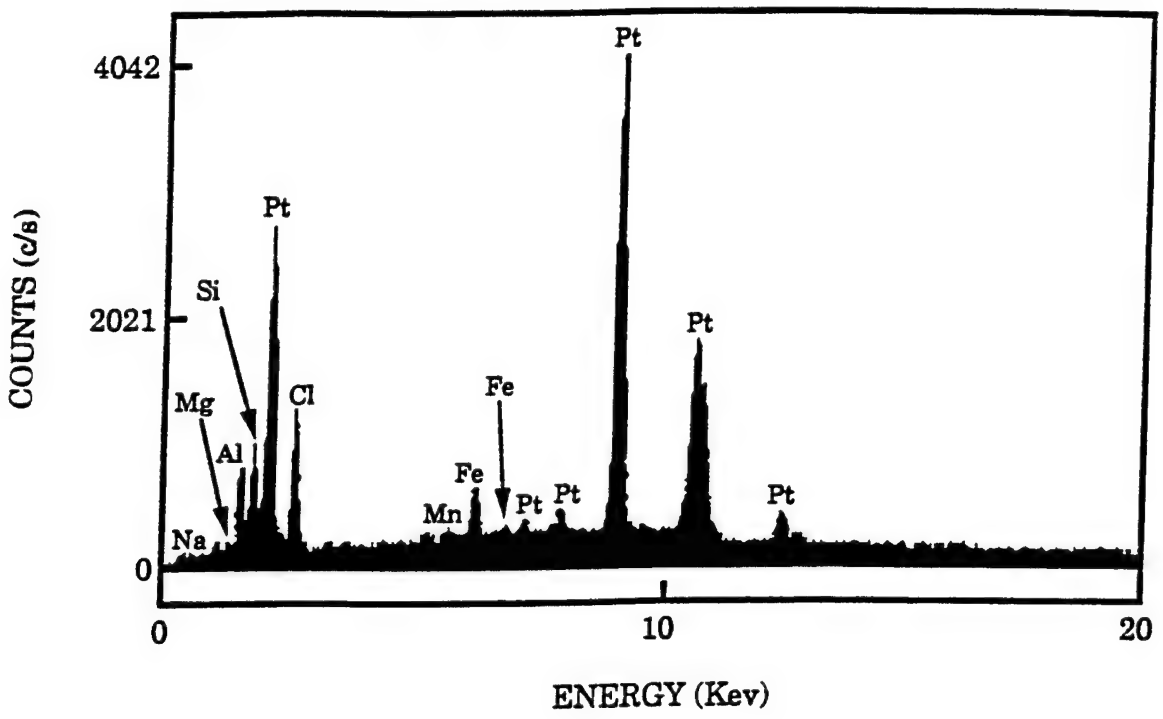

Figure 3.4 EDAX data for a biofilmed platinum electrode exposed in seawater for 2 months. Note the iron and manganese peaks.

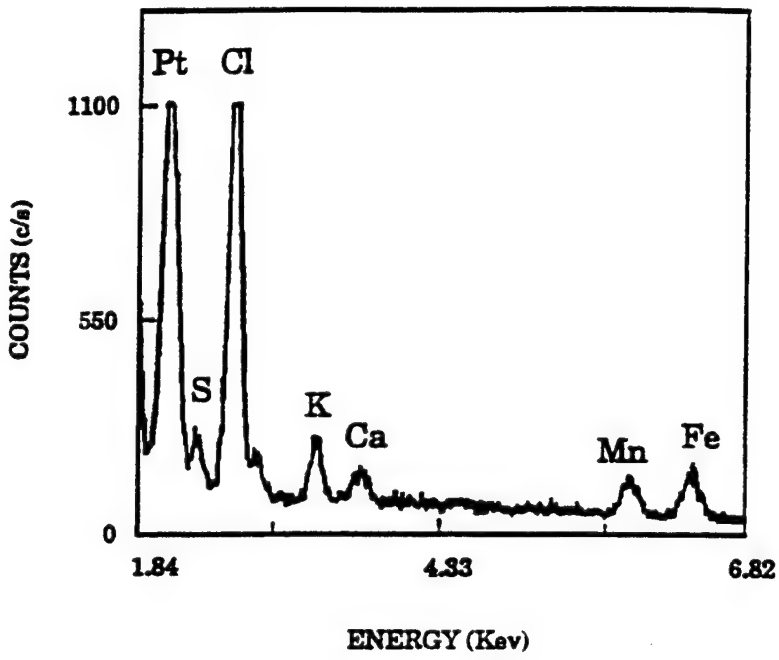

Figure 3.5 EDAX data for a biofilmed platinum electrode exposed in seawater for a period of 4 months.

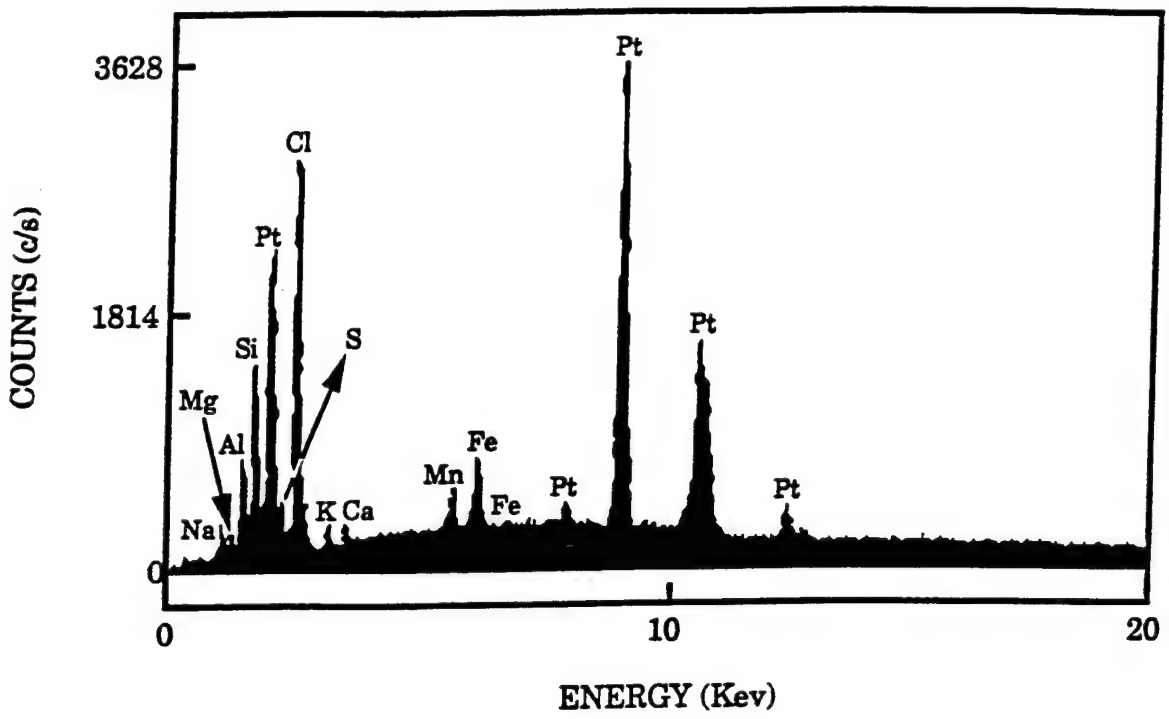

Figure 3.6 EDAX data for a biofilmed platinum electrode exposed in seawater for a period of 5 months.

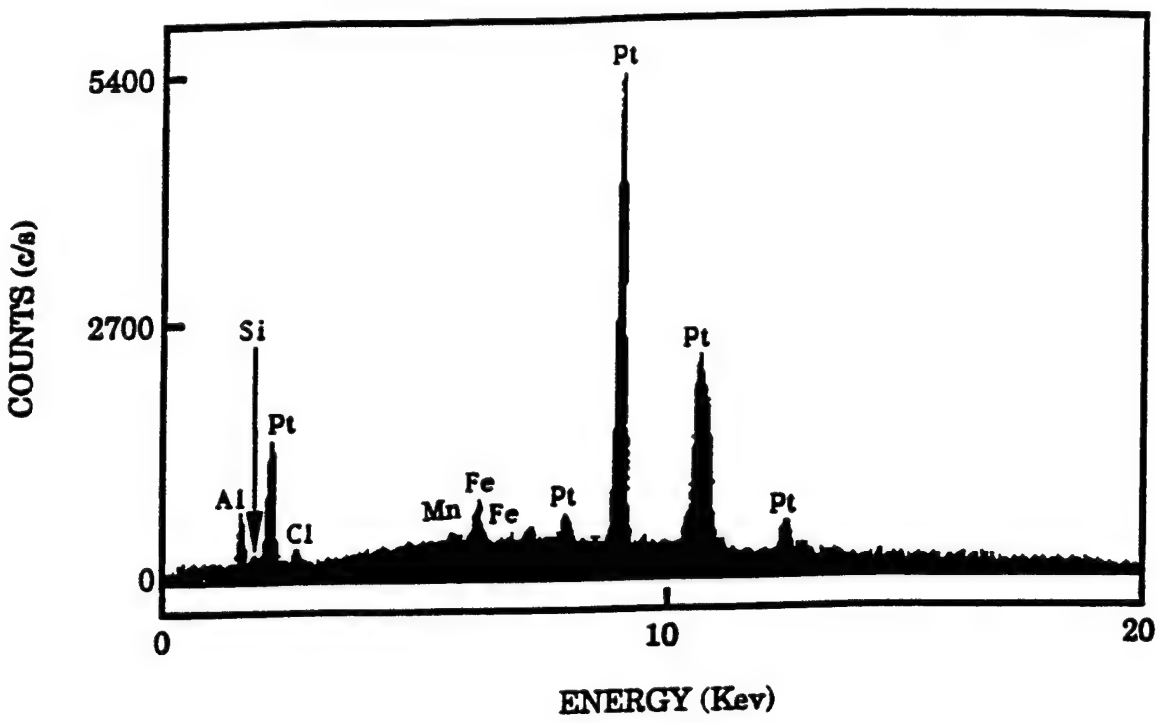

Figure 3.7 EDAX data for a biofilmed platinum electrode exposed in seawater for a period of 24 months.

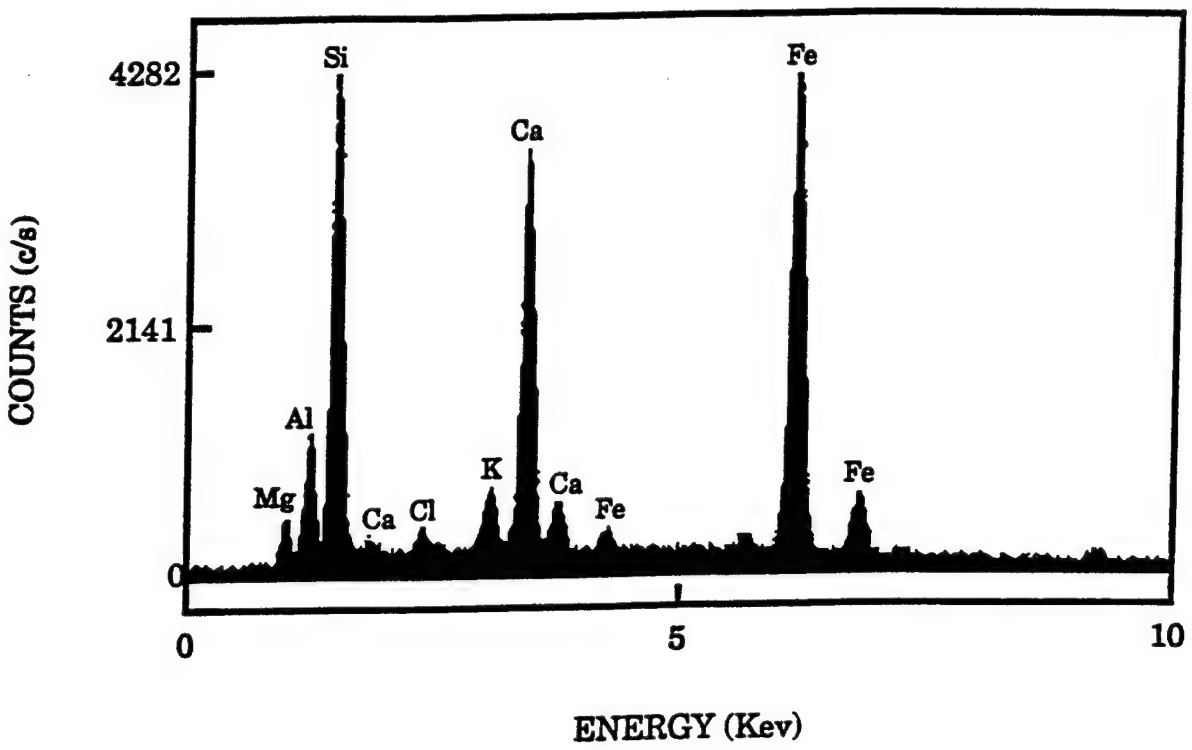

Figure 3.8 EDAX data for a small particle (9 mm across) found in a biofilm developed over platinum.

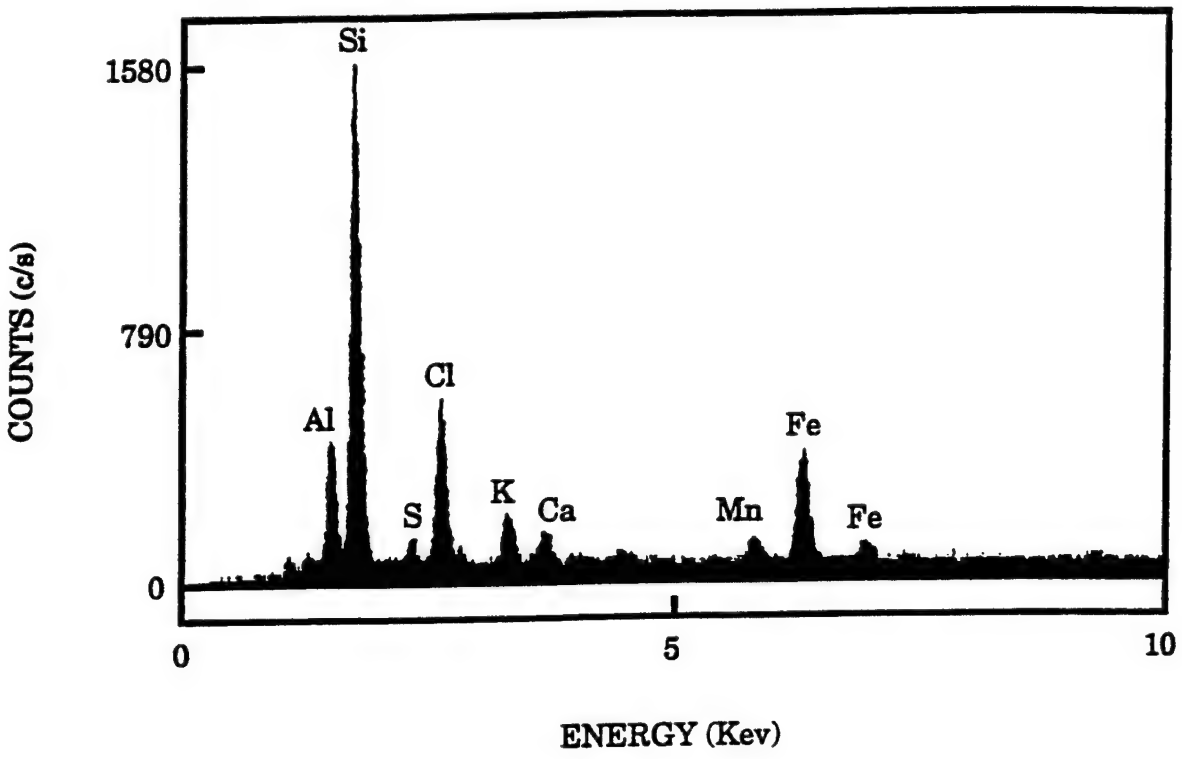

Figure 3.9 EDAX data for a larger particle (20 to 47 mm across) found in a biofilm developed over platinum.

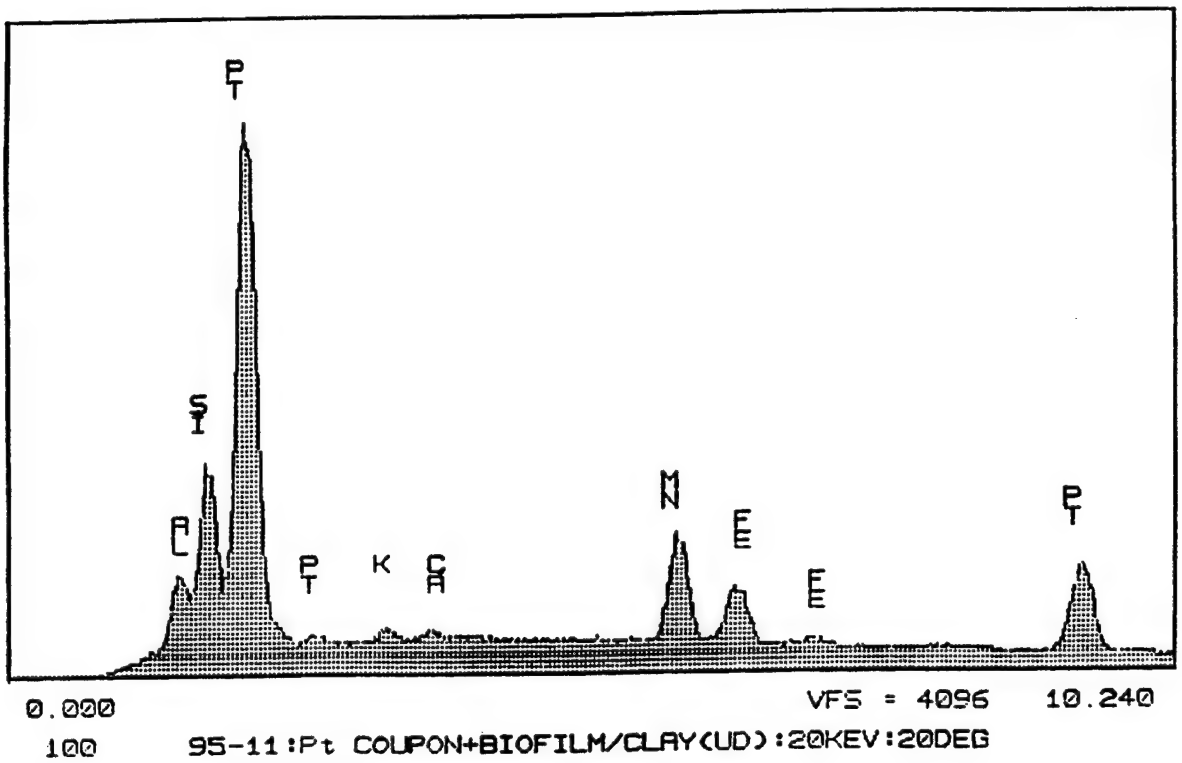

Figure 3.10 ESEM and EDAX data for a biofilmed platinum electrode exposed for a period of 15 months in Delaware Bay seawater. Note the iron and manganese peaks.

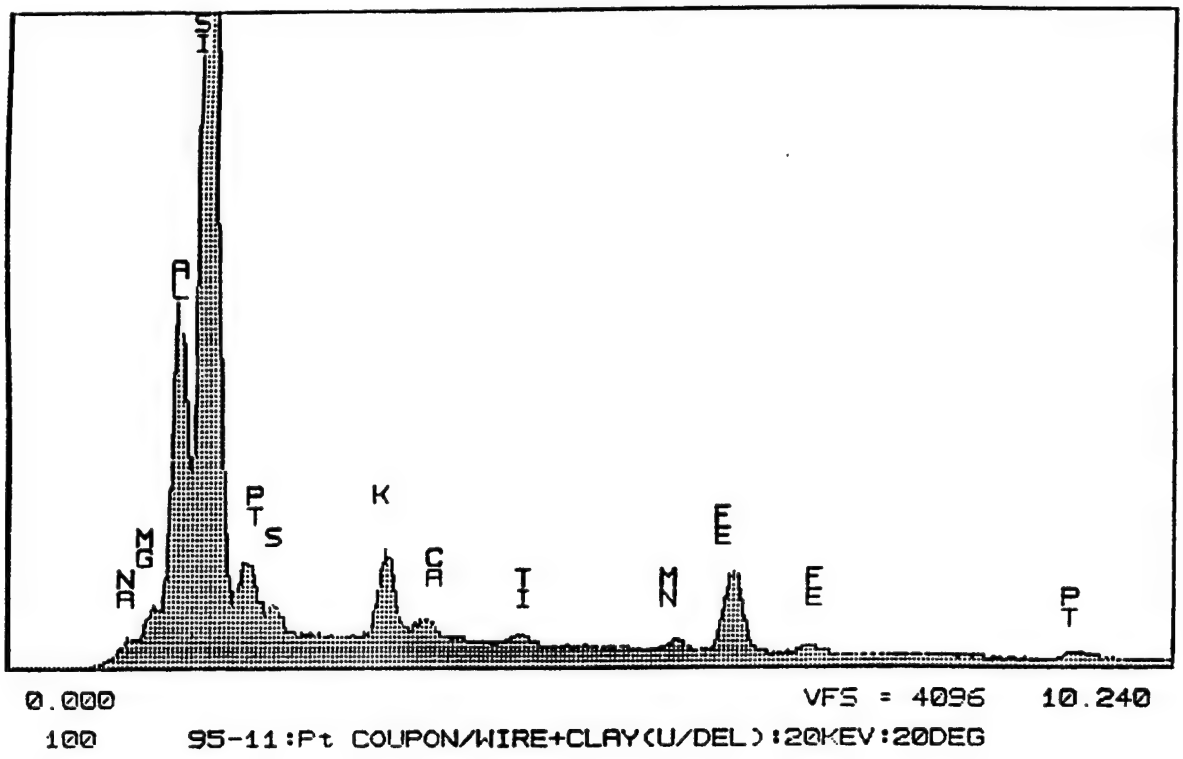

Figure 3.11 ESEM and EDAX data for a biofilmed platinum electrode exposed for a period of 15 months in Delaware Bay seawater. Note the iron and manganese peaks.

DISTRIBUTION LIST

Dr. A. John Sedriks
Materials Division, Code 1131M
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5660

Defense Technical Information Center
8725 John J. Kingman Rd., Suite 0994
Ft. Belvoir, VA 22060-6218

Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5660
ATTN: Code 332

Naval Research Laboratory
4555 Overlook Ave., S.W.
Washington, DC 20375
ATTN: Code 6000
Code 6300

Naval Surface Warfare Center -
Carderock Division
Bldg. 60, Corrosion Branch
Bethesda, MD 20084
ATTN: Code 613

Naval Postgraduate School
Monterey, CA 93940
ATTN: Mechanical Engineering Dept.

Naval Sea Systems Command, Dept.
of Navy, 2531 Jefferson Davis Highway
Arlington, VA 22242
ATTN: Code 03M

Naval Air Warfare Center
Aerospace Materials Division
Code 4.3.4, MS 3 , Building 2188
Patuxent River, MD 20670-5304
ATTN: Code 4.3.4

Naval Facilities
Engineering Service Center
Port Hueneme, CA 94043
ATTN: Materials Div.

Naval Surface Warfare Center
Carderock Division
Bethesda, MD 20084
ATTN: Library

Naval Underwater Warfare Ctr.
Newport, RI 02840
ATTN: Library

Naval Air Warfare Center
Weapons Division
China Lake, CA 93555-6001
ATTN: Library

NASA
Lewis Research Center
21000 Brookpark Road
Cleveland, OH 44135
ATTN: Library

National Institute of
Standards and Technology
Gaithersburg, MD 20899
ATTN: Metallurgy Division

Naval Command, Control and
Ocean Surveilance Center
R&D Division
San Diego, CA 92152-5000
ATTN: Library

Office of the Assistant Commander
HQ Marine Corps
2 Navy Annex
Washington, DC 20380-1775
ATTN: Scientific Advisor

Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709
ATTN: Metallurgy & Ceramics Program

Air Force Office of Scientific Research
Building 410
Bolling Air Force Base
Washington, DC 20332
ATTN: Electronics & Materials
Science Directorate

NASA Headquarters
Washington, DC 20546
ATTN: Code RN

Naval Surface Warfare Center
Port Hueneme Division
4363 Missile Way
Port Hueneme, CA 93043-4307
ATTN: Library

Metals Information Analysis Center
Purdue University
2595 Yeager Road
West Lafayette, IN 47906

Naval Facilities Engineering Command
Alexandria, VA 22331
ATTN: Code 03

Oak Ridge National Laboratory
Metals and Ceramics Division
P.O. Box X
Oak Ridge, TN 37380

Los Alamos Scientific Laboratory
P.O. Box 1663
Los Alamos, NM 87544
ATTN: Report Librarian

Argonne National Laboratory
Metallurgy Division
P.O. Box 229
Lemont, IL 60439

Brookhaven National Laboratory
Upton, Long Island, NY 11973
ATTN: Research Library

Lawrence Berkeley Laboratory
1 Cyclotron Rd.
Berkeley, CA 94720
ATTN: Library

Office of Naval Research Asian Office
Unit 45002
APO Area Pacific 96337-0007

Professors G. H. Meier and F. S. Pettit
Department of Materials Science
and Engineeering
848 Benedum Hall
University of Pittsburgh
Pittsburgh, PA 15261

Professor Gordon P. Bierwagen
North Dakota State University
Department of Polymers and Coatings
Box 5227
Fargo, ND 58105

Professor H. W. Pickering
The Pennsylvania State University
209 Steidle Building
University Park, PA 16802

Professor D. J. Duquette
Department of Metallurgical Engineering
Rensselaer Polytechnic Institute
Troy, NY 12181

Professor D. Tomanek
Michigan State University
Department of Physics and Astronomy
East Lansing, MI 48824-1116

Dr. M. W. Kendig
Rockwell International Science Center
1049 Camino Dos Rios
P.O. Box 1085
Thousand Oaks, CA 91360

Professor R. A. Rapp
Department of Metallurgical Engineering
The Ohio State University
116 West 19th Avenue
Columbus, OH 43210-1179

Dr. R. D. Granata
Sinclair Laboratory #7
Lehigh University
Bethelam, PA 18015

Dr. G. D. Davis
Martin Marietta Laboratories 848
1450 South Rolling Road
Baltimore, MD 21227-3898

Dr. S. M. Lipka
Department of Ocean Engineering
Florida Atlantic University
Boca Raton, FL 33431-0991

Professor D. D. Macdonald
The Pennsylvania State University
517 Deike Building
University Park, PA 16802

Dr. B. G. Pound
SRI International
333 Ravenswood Avenue
Menlo Park, CA 94025

Professor C. R. Clayton
Department of Materials Science
and Engineering
State University of New York -
Stony Brook
Stony Brook, NY 11794

Dr. J. W. Oldfield
Cortest Laboratories Ltd.
23 Shepherd Street
Sheffield, S3 7BA
UNITED KINGDOM

Ms. D. M. Aylor
Code 613
Naval Surface Warfare Center
Annapolis, MD 21402-5067

Professor K. Sieradzki
Department of Mechanical
and Aerospace Engineering
Arizona State University
Tempe, AZ 85287-6106

Dr. P. S. Pao
Code 6326
Naval Research Laboratory
Washington, DC 20375-5343

Dr. W. P. Allen
United Technologies Research Center
East Hartford, CT 06108

Dr. B. A. Shaw
Department of Engineering Science
and Mathematics
207 Hallowell Building
The Pennsylvania State University
University Park, PA 16802-1484

Dr. P. Cox
SRI International
333 Ravenswood Avenue
Menlo Park, CA 94025-3493

Dr. R. E. Ricker
National Institute of Standards
and Technology
Building 223, Room B-266
Gaithersburg, MD 20899

Dr. F. B. Mansfeld
Department of Materials Science
University of Southern California
University Park
Los Angeles, CA 90089-0241

Professor R. E. White
Department of Chemical Engineering
University of South Carolina
Columbia, SC 29208

Professor R. A. Buchanan
Department of Materials Science
and Engineering
University of Tennessee
Knoxville, TN 37996-2200

Dr. B. J. Little
NRL Detachment
Building 1105, Room D415
Stennis Space Center, MS 39529-5004

Professor M. E. Orazem
Department of Chemical Engineering
University of Florida
Gainesville, FL 32611

Professor J. O'M. Bockris
Department of Chemistry
Texas A&M University
College Station, TX 77843

Dr. V. S. Agarwala
Code 6062
Naval Air Warfare Center
Warminster, PA 18974-0591

Professor R. C. Newman
UMIST
Corrosion and Protection Center
P.O. Box 88
Manchester M60 1QD
UNITED KINGDOM

Dr. R. Brown
Department of Chemical Engineering
University of Rhode Island
Kingston, RI 02881-0805

Dr. J. Jones-Meehan
Code R301
Naval Surface Warfare Center
10901 New Hampshire Avenue
Silver Spring, MD 20903

Dr. P. Natishan
Code 6322
Naval Research Laboratory
Washington, DC 20375-5343

Dr. R. L. Jones
Code 6170
Naval Research Laboratory
Washington, DC 20375-5342

Dr. Stephen C. Dexter

Dr. George W. Luther, III

Kimberly Habiger